Micro-computed tomography for the quantification of blocked fibers in hemodialyzers

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A novel technique based on micro-CT scanning is developed to quantify coagulation in fibers of hemodialyzers. This objectivation is needed to allow accurate assessment of thrombogenicity of dialyzers used during hemodialysis, for example when comparing different strategies to avoid coagulation and/or fiber blocking. The protocol allowed imaging at a resolution of 25 µm, making it possible to count the open, non-coagulated fibers in a non-invasive way. In 3 fresh, non-used FX600 hemodialyzers, patent fiber counts were extremely consistent (10748 ± 2). To illustrate the potential of this technique, different dialysis parameters currently used as surrogates for fiber blocking were evaluated during 20 hemodialysis sessions. After dialysis, the FX600 dialyzers were visually scored for clotting, dried and subsequently weighed and scanned. The number of patent fibers (10003 [8763,10330], range 534–10692) did not correlate with any of the recorded surrogate parameters. Micro-CT scanning is a feasible, objective, non-invasive, accurate and reproducible tool for quantification of the degree of fiber blocking in a hemodialyzer after use, making it a potential gold standard for use in studies on fiber blocking during renal replacement therapies.
The present study examines the feasibility and accuracy of micro-computed tomography (micro-CT) to quantify the number of blocked fibers. As illustration, the number of blocked fibers as measured by micro-CT is compared to other surrogate parameters used to assess fiber blocking.

**Results**

Micro-CT scanning allowed to acquire high quality images of cross-sections of all scanned dialyzers. Individual capillary fibers could easily be distinguished and their total number quantified. Fiber counting in corresponding cross-sections of the 3 non-used dialyzers resulted in almost exactly the same number of fibers (e.g. 10748 ± 2 in the reference slice), as is expected taking into account the high consistency of the manufactural process of bundling fibers in individual dialyzer capsules.

Figure 1 shows images representing different levels of coagulation in different used dialyzers. Complete circuit blocking because of dialyzer clotting did not occur during the sessions of the current experiments, but the 20

![Figure 1. Representative CT images of dialyzer cross-sections (reference slice) showing different levels of coagulation.](image-url)
dialyzers under study represented a broad spectrum of coagulation activation, as reflected by the wide range (534 to 10692) of fibers remaining open after the dialysis session. Table 1 shows the number of counted open fibers in the non-used and used dialyzers and this in different cross-sections and for different thresholds of open fiber surface areas. Good similarity in counts of open fibers remained when images were assessed using different thresholds to define an ‘open fiber’ (i.e. difference $\leq 1.7\%$), showing the robustness and validity of the technique. Differences in the number of open fibers as counted in different cross-sections in the potting were within the clinical relevance ($\leq 6.1\%$), endorsing the reliability of the technique.

Associations were found between the number of open fibers and the post-dialysis dry mass of the dialyzers ($R^2 = 0.62$), and the visual clotting scores of the dialyzers ($R^2 = 0.41$) and venous chambers ($R^2 = 0.34$). However, none of the online available parameters as indicated on the dialysis machine (i.e. pressures, blood volume monitoring and online clearance monitoring) as well as the post dialysis registered rinse-back volume were associated with the number of open fibers (Table 2 and Fig. 2).

**Discussion**

We demonstrate the feasibility to use a self-developed micro-CT technique to assess objectively and in an operator-independent manner the blocking of individual fibers in hemodialyzers with great accuracy and repeatability. Using this technique, we demonstrate that currently used parameters, based on data derived from the dialysis monitor, visual scoring or post-dialysis mass of the dialyzer are not representative to assess the degree of fiber blocking.

The circulation of blood through extracorporeal devices such as hemodialyzers activates the coagulation cascade, which might result in clotting and obstruction of the circuit. In hemodialysis, this progressive blocking of fibers within the dialyzer results in a progressive decrease of dialysis performance, and potential loss of blood when the circuit becomes completely obstructed. The degree of activation of the coagulation cascade depends on the biocompatibility of the dialyzer and the coagulability of the blood. Biocompatibility is in itself a combination of design, for example geometry to avoid turbulence, blood stagnation and shear stress, and the type of materials used\cite{19,20}. Coagulability depends upon patient factors, for example presence of inflammation or coagulation disorders, and can be influenced by applying an anticoagulant strategy during the dialysis session. This should however be done with caution, as too much anticoagulation might induce bleeding and death of the patient.
Different designs of filters and anticoagulant strategies have been developed, but comparing the balance of efficacy and safety between different designs and strategies is hampered by the lack of a well-established and objective tool to evaluate the resulting degree of filter blocking in the hemodialyzer. There is thus need for a gold

| Dialyzer | #fibers ref slice 70% threshold | dialyzer dry mass (g) | visual scoring dialyzer | visual scoring venous chamber | Rinse back volume (mL) | arterial pressure pre-post (mmHg) | venous pressure pre-post (mmHg) | TMP post-pre (mmHg) | BVM pre-post (%) | OCM pre-post (mL/min) |
|----------|---------------------------------|----------------------|------------------------|-----------------------------|------------------------|-------------------------------|-------------------------------|----------------------|-----------------|-----------------------|
| 1        | 5443                            | 224.0                | 4                      | 3                           | 220                    | 25                            | 5                             | 160                  | 7               | 28                    |
| 2        | 7632                            | 222.5                | 3                      | 2                           | 280                    | 0                             | −45                           | 80                   | 9               | 31                    |
| 3        | 10328                           | 142.0                | 1                      | 1                           | 280                    | 10                            | 20                            | 20                   | 30              | 4                     |
| 4        | 10246                           | 176.0                | 2                      | 1                           | 270                    | 10                            | −105                          | 55                   | 11              | 19                    |
| 5        | 10335                           | 187.5                | 2                      | 2                           | 270                    | 30                            | 15                            | 20                   | 6               | 6                     |
| 6        | 10228                           | 187.5                | 2                      | 2                           | 270                    | −25                           | 0                             | 65                   | 6               | 5                     |
| 7        | 8327                            | 202.5                | 3                      | 2                           | 260                    | 25                            | 10                            | 35                   | 5               | 4                     |
| 8        | 534                             | 283.0                | 4                      | 3                           | 360                    | 60                            | −35                           | 75                   | 20              | 66                    |
| 9        | 10041                           | 171.0                | 1                      | 1                           | 260                    | 55                            | −20                           | 10                   | 12              | −9                    |
| 10       | 9451                            | 212.0                | 1                      | 1                           | 270                    | 15                            | 40                            | 65                   | 10              | 17                    |
| 11       | 10234                           | 192.0                | 2                      | 2                           | 260                    | 65                            | 30                            | 60                   | 9               | 13                    |
| 12       | 6066                            | 240.0                | 3                      | 3                           | 270                    | −5                            | 75                            | 15                   | 3               | 6                     |
| 13       | 16692                           | 157.5                | 1                      | 2                           | 270                    | 15                            | 5                             | 25                   | 7               | 0                     |
| 14       | 10587                           | 137.0                | 2                      | 2                           | 290                    | 10                            | 0                             | 50                   | 5               | 3                     |
| 15       | 8921                            | 158.0                | 4                      | 2                           | 260                    | −10                           | 5                             | 110                  | 5               | 15                    |
| 16       | 10351                           | 202.0                | 2                      | 1                           | 310                    | −35                           | 5                             | 5                    | 2               | 2                     |
| 17       | 8998                            | 209.0                | 4                      | 1                           | 260                    | −10                           | 15                            | 125                  | 6               | 8                     |
| 18       | 10465                           | 167.0                | 2                      | 2                           | 270                    | 20                            | 20                            | 70                   | 13              | 10                    |
| 19       | 9965                            | 217.0                | 3                      | 3                           | 250                    | 65                            | 45                            | 35                   | 8               | 92                    |
| 20       | 9752                            | 218.5                | 3                      | 1                           | 260                    | 35                            | 20                            | 35                   | 13              | 49                    |

| R² (with #fibers): | 0.62* | 0.41* | 0.34* | 0.20 | 0.04 | 0.01 | 0.14 | 0.18 | 0.15 |

Table 2. Absolute values as well as regression coefficients (R²) of the associations of the number of open fibers with the post dialysis measurements and scorings, and with the interdialytic changes in registered machine parameters in the 20 dialysis sessions. *p < 0.05; #number; TMP: transmembrane pressure; BVM: blood volume monitoring; OCM: online clearance monitoring.

Figure 2. Associations between the number of fibers and a selection of different registered parameters: dialyzer dry mass (panel A), visual scoring of dialyzer (panel B), post minus pre dialysis TMP (panel C), and pre minus post dialysis OCM (panel D).

Different designs of filters and anticoagulant strategies have been developed, but comparing the balance of efficacy and safety between different designs and strategies is hampered by the lack of a well-established and objective tool to evaluate the resulting degree of filter blocking in the hemodialyzer. There is thus need for a gold
standard to assess and compare thrombogenicity of different dialyzers and/or anticoagulation strategies. Ideally, such technique quantifies the patency of the individual fibers in the complete dialyzer, to assess the impact of coagulation on the dialyzer performance. The technique should be repeatable, accurate and operator-independent (objective). The here presented micro-CT scanning technique fulfills these criteria. It allows consistent and reliable counting of open versus blocked fibers in an accurate and repeatable way, as demonstrated by the agreement of measurements at different cross-sections of the dialyzer. In addition, this high accuracy is independent of the threshold chosen to define patency of fibers.

The present technique offers multiple advantages over the currently available methods to assess the degree of fiber blocking. So far, visual assessment has been most frequently promoted to assess coagulation of dialyzers. In this technique, a visual assessment of the discoloration of the fibers from white to red by residual (clotted and blocking) blood is done to estimate the degree of fiber blocking within the dialyzer. Despite existence of tools to enhance standardization, such as calibrated reference pictures, this simple method remains operator-dependent. This operator-dependency can theoretically be decreased by using computer analysis of pictures of the dialyzer rather than visual assessment by the human eye. However, still the technique allows only assessment of fibers at the outside of the dialyzer, as more internal fibers cannot be visualized. As coagulation is not homogenous, this can result in big discrepancies between estimated and true degree of coagulation. This is more of importance as coagulation can occur at specific areas exactly because of the design of the dialyzer, for example at places of high turbulence, or stagnation of flow. The lack of capacity to assess the complete cross-section by visual assessment can thus lead to misinterpretation of the advantage/disadvantage of certain designs or strategies. Micro-CT evaluation is performed on complete sections of the dialyzer, allowing assessment of all fibers, irrespective of their location. Last, visual scoring of clotting can be hampered by non-transparent fiber and housing design, and can thus not be used in all types of dialyzers while the presented micro-CT technique is independent of the type of housing. In our study, there was a relatively good association between the visual score of the hemodialyzer and the number of blocked fibers ($R^2 = 0.36$) (Table 2 and Fig. 2 panel B). However, the point prevalence power of the visual score to predict the number of open fibers in individual dialyzers was disappointing. For example, a visual score of 3 corresponded with 6000 in one and 10000 open fibers in another dialyzer, whereas 10000 open fibers resulted in a visual score of 1 in one and 3 in another dialyzer (Fig. 2 panel B), making that visual scoring is completely not reliable at the level of the individual patient.

Dialyzer mass after post dialysis rinsing could be used as a proxy of blocked fibers. This method is based on the postulate that it is impossible to remove debris and blood from blocked fibers, so that more mass corresponds with more blocked fibers. In our experiments, post dialysis dialyzer mass corresponded best of all parameters with the number of open fibers as assessed by micro-CT, with an $R^2$ of 0.62 (Table 2 and Fig. 2 panel A). However, also here, the point prevalence predictive power was disappointing and insufficient to accept post dialysis dialyzer mass as an acceptable surrogate for degree of fiber blocking.

Laboratory markers for coagulation activation have been used to assess degree of coagulation during a hemodialysis session. However, there is a lack of standardization of how these tests should be performed and how results should be interpreted. More important, the results of these tests are representing coagulation in the complete system, including the patient. As such, it is unclear from these tests where exactly the coagulation cascade has been activated, either in the patient, for example due to inflammation, or in the dialyzer. As a consequence, these tests are less informative about the impact of dialyzer design or anticoagulation strategy on the blocking of fibers.

In many centers, parameters that are routinely available from the dialysis machine, such as venous pressure and estimated transmembrane pressure are being used to assess the degree of fiber blocking. From the regression analyses, we found that none of these monitor derived parameters accurately reflects degree of coagulation. Pre-dialyzer pressure and measured transmembrane pressure potentially would perform better, but none of these parameters is provided on the presently commercially available dialysis machines.

Also the volume needed to achieve clean rinse-back, as determined by a sensor on the venous blood line or chamber, seems not to be able to predict the number of blocked fibers. This corresponds with the observation that visual inspection of blood lines and chambers does not necessarily reflect coagulation within the dialyzer.

This study also showed for the first time that online clearance monitoring (OCM), claimed to be an indicator of dialysis performance, does not correspond with the degree of fiber blocking during dialysis. For example, in a dialyzer ending up with only 534 patent fibers out of more than 10000 at start, (as shown in Fig. 1, lower right image), OCM values were only decreased by 28% after the 4 hour dialysis session. Although dialyzer clotting is a dialyzer ending up with only 534 patent fibers out of more than 10000 at start, (as shown in Fig. 1, lower right image), OCM values were only decreased by 28% after the 4 hour dialysis session. Although dialyzer clotting is
 Patients and Methods

This single center, open-label, non-controlled prospective trial included twenty adult patients (11 male) undergoing 245 ± 20 min maintenance hemodialysis (pressure controlled on-line postdilution hemodiafiltration, replacement fluid 20 ± 4 L). The median age was 75 [69–79]. Median weight was 65 [58–72] kg. Diabetic and hypertensive nephropathy were the most frequent underlying diseases. Sixty percent of the patients received anti-platelet therapy. Table 3 lists the patient's clinical characteristics and baseline laboratory findings.

The protocol was approved by the ethics committee of the Ghent University Hospital, and written informed consent was obtained from all included patients or their legal representatives (EC 2016/0908–B670201629247).

Dialysis and anticoagulation.

All dialysis treatments were performed in accordance with the relevant guidelines and regulations. Patients received their standard Low-Molecular-Weight Heparin (Enoxaparin, Sanofi Belgium or Tinzaparin, Leo Pharma, Belgium) anticoagulation at the beginning of the dialysis session. All sessions were performed with single-use high flux FX600 Cordiax® polysulfone dialyzers with a surface area of 1.6 m² on 5008 dialysis machines (Both Fresenius Medical Care, Bad Homburg, Germany). Blood flow was maintained between 200 and 350 ml/min (297 ± 43), while ultrafiltration was set according to the patient's interdialytic weight gain and clinical status (509 ± 194 ml/h). In all dialysis sessions, double-needle vascular access was achieved through a native arteriovenous fistula or a well-functioning double lumen tunneled central venous catheter.

Online dialysis machine parameters.

Different dialysis parameters as reported by the dialysis machine were registered every 30 min: i.e. venous and arterial pressure, transmembrane pressure (TMP), blood volume monitoring (BVM) and online clearance monitoring (OCM). For further regression analyses, absolute changes, calculated as post versus predialysis values, were used. Rinse-back volume, representing the volume of dialysate needed to restore all extracorporeal blood back to the patient at the end of the session was registered from the dialysis monitor.

| Cause of ESRD no. (%) | Median [Q1; Q3] and (min; max) or n (%) |
|-----------------------|----------------------------------------|
| Diabetic/hypertensive nephropathy | 7 (35%) |
| Post-Renal Causes | 3 (15%) |
| Glomerulonephritis | 4 (20%) |
| Others | 6 (30%) |

| Co-existing illnesses no. (%) | Median [Q1; Q3] and (min; max) or n (%) |
|------------------------------|----------------------------------------|
| Smoking | 2 (10%) |
| History of cancer | 7 (35%) |
| Diabetes | 6 (30%) |
| Thromboembolic Venous Disease | 5 (25%) |

| Antithrombic therapy—no. (%) | Median [Q1; Q3] and (min; max) or n (%) |
|------------------------------|----------------------------------------|
| Aspirine (monotherapy) | 11 (55%) |
| Clopidogrel (monotherapy) | 0 |
| Aspirine and Clopidogrel | 1 (5%) |
| Vitamin K antagonist | 3 (15%) |

| Dialysis LMWH anticoagulation | Median [Q1; Q3] and (min; max) or n (%) |
|------------------------------|----------------------------------------|
| No anticoagulation | 1 (5%) |
| Enoxaparin 20 mg | 1 (5%) |
| Enoxaparin 40 mg | 4 (20%) |
| Enoxaparin 60 mg | 4 (20%) |
| Tinzaparin 2500 U | 2 (10%) |
| Tinzaparin 3500 U | 5 (25%) |
| Tinzaparin 4500 U | 3 (15%) |

| Clotting Parameters (mean ± SD) | Median [Q1; Q3] and (min; max) or n (%) |
|---------------------------------|----------------------------------------|
| Hemoglobin, g/dl | 9.8 [8.7–11.8] (7.9–13.5) |
| Hematocrit, % | 31.0 [27.7–36.0] (22.8–41.6) |
| Platelets, x 10E3/µL | 179 [115–255] (43–431) |
| Prothrombin time (PT) | 86 [69–102] (21–115) |
| International Normalized Ratio (INR) | 1.1 [1.0–1.3] (0.9–3.6) |

| Access | Median [Q1; Q3] and (min; max) or n (%) |
|--------|----------------------------------------|
| Arteriovenous fistula | 7 (35%) |
| Permanent dialysis catheter | 13 (63%) |

Table 3. Patient characteristics and baseline laboratory data.
Dialyzer and venous chamber scoring. Hemodialyzers and blood lines were removed immediately once the post-dialysis standard rinsing procedure had been completed. Subsequently, a member of the nursing staff and a clinician independently scored the extent of clotting in the dialyzer and the venous chamber in a semi-quantitative way using a reference scoring system\(^\text{10}\). Dialyzers were graded having no signs of clotting (1), few dark red fibers (2), less than 50% dark red fibers (3) or overall redness with >50% dark red fibers (4). Thrombus formation in the air bubble catcher (venous chamber) was quoted using a four graded scale: no detectable clotting (1), minimal clot formation (2), clots up to 5 cm but dialysis still possible (3), and complete occlusion of the air bubble catcher (4) (Table 4).

Next, in an in vitro setting, continuous subtle positive pressure ventilation was applied to the dialyzer both through the blood inlet as through the dialysate outlet for 24 hours, to dry the dialyzer membrane. Finally, dialyzer dry mass was measured using a precise scale (CT 6000, Ohaus\(^\text{19}\), USA - calibration by Eldon Enterprises France).

Micro-CT scanning. Dialyzer fiber clotting was visualized using a 3D CT scanning technique on micrometer resolution. HECTOR is a High Energy CT scanner Optimized for Research\(^\text{22}\), built by the Ghent University Centre for X-ray Tomography (UGCT) in collaboration with the UGCT spin-off company XRE (Gent, Belgium). In front of the X-ray source, the dialyzer was mounted vertically on a precision rotation stage, and radiographies were recorded over 360° with an angular interval of 0.15°. Scan conditions were optimized to maximize the signal-to-noise ratio based on the sample size and structure, and the scanner properties. The tube voltage was set at 80 kV, at a power of 20 Watts, the maximal power that allowed imaging at a resolution of 25 µm. A total of 2401 projections were recorded with 500 ms exposure each, resulting in a total exposure time of 20 minutes. Acquired images at 0 (projection 1) and 360° (projection 2401) were compared to exclude movement of the hemodialyzer during the scanning process. Reconstruction of the raw projection data is performed with the Octopus Reconstruction software package, licensed by XRE\(^\text{23}\). This resulted in two types of images as presented in Fig. 3: fibers are visible as rings for cross-sections in the free fiber bundle (left panel), and as black dots for cross-sections in the dialyzer potting (right panel), a polyurethane sealing near the blood inlet and outlet, fixing the ends of the capillary fibers.

Fiber counting and coagulation quantification. Fibers were counted in the central cross-section of the dialyzer outlet potting (reference slice) since this region offers optimal visualization of air inflated open fibers, appearing as black dots (Fig. 3–right panel). These dots can easily be counted in a computer-based way, in contrast to the use of cross-sectional images taken out of the potting showing fibers as white cylinders (Fig. 3–left panel).

| Clotting score (dialyzer) | Number of dialyzers (%) |
|--------------------------|-------------------------|
| 1 Clean filter           | 4 (20)                  |
| 2 Few dark red fibers    | 7 (35)                  |
| 3 <50% dark red fibers   | 5 (25)                  |
| 4 >50% dark red fibers   | 4 (20)                  |

Table 4. Visual clotting scores.

Figure 3. CT images of cross-sections in the same used dialyzer halfway the dialyzer (left panel) and at the central cross-section in the outlet potting (right panel). Central fibers are open and peripheral fibers are blocked. Sections in the potting offer optimal visualization of air inflated open fibers, appearing as black dots.
The non-coagulated fibers (i.e. black dots) were counted using the Fiji image processing toolkit of ImageJ analysis software.2426 [ImageJ 1.51 H, NIH, Bethesda, USA], an open-source platform for biological-image analysis.27 To ameliorate selection and count of the patent fibers, the brightness of each individual image was optimized using image thresholding.27 The region of interest (ROI) was selected bordering the entire fiber bundle to avoid count of any disturbances outside the fiber bundle. Watershed segmentation was employed to distinguish sticky fibers.28 Image particles with a circularity of 0.5–1 were selected (Fig. 4–panel C).

Based on the reference images of the non-used dialyzers, only fibers with a surface area of >70% (threshold) of the cross section of a non-used fiber were considered to be open. This correlates with a surface area of 0.0204 mm². Validity and reliability of the method were checked, the former by comparing the impact of different arbitrary thresholds (60 and 80% open fiber) to define open fibers, the latter by performing counts in a cross-section 100 slices (i.e. 2.5 mm) proximal of the reference slice.

**Statistical analysis.** Statistical analyses were performed using SPSS (version 24, SPSS Inc, Chicago, USA). Continuous variables were summarized as mean ± SD if normally distributed (normality was checked with Shapiro-Wilk test); otherwise median value with interquartile range [IQR] were reported. Categorical variables were expressed as frequencies and percentages. Regression analyses were performed (R²) with the number of open fibers as independent variable and the dialysis parameters as dependent variables.

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Author Contributions
F.V.O., W.V.B., A.D. and S.E. developed the study design. F.V.O. and S.E. performed the clinical part of the study. M.D. and M.B. performed the micro C.T. scanning and the image reconstruction. F.V.O. and S.E. performed the fiber counting and statistical analysis. F.V.O., S.E. and W.V.B. wrote the main manuscript.

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