

Cross-talk between inflammation, coagulation/fibrinolysis and vascular access in hemodialysis patients

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Abstract: This work aimed to study the association between fibrinolytic/endothelial cell function and inflammatory markers in chronic kidney disease (CKD) patients undergoing hemodialysis (HD) and recombinant human erythropoietin (rhEPO) therapies, and its relationship with the type of vascular access (VA) used for the HD procedure. As fibrinolytic/endothelial cell function markers we evaluated plasminogen activator inhibitor type-1 (PAI-1), tissue plasminogen activator (tPA) and D-dimers, and as inflammatory markers; C-reactive protein (CRP), soluble interleukin (IL)-2 receptor (s-IL2R), IL-6 and serum albumin levels. The study was performed in 50 CKD patients undergoing regular HD, 11 with a central venous dialysis catheter (CVC) and 39 with an arteriovenous fistula (AVF), and in 25 healthy controls. Compared to controls, CKD patients presented with significantly higher levels of CRP, s-IL2R, IL-6 and D-dimers, and significantly lower levels of PAI-1. The tPA/PAI-1 ratio was significantly higher in CKD patients. We also found statistical significant correlations in CKD patients between D-dimers levels and inflammatory markers: CRP, albumin, s-IL2R and IL-6. When comparing the two groups of CKD patients, we found that those with a CVC presented statistically significant lower levels of hemoglobin concentration and albumin, and higher levels of CRP, IL-6, D-dimers and tPA. Our results showed an association between fibrinolytic/endothelial cell function and increased inflammatory markers in CKD patients. The increased levels of D-dimer, tPA and inflammatory markers in CKD patients using a CVC, led us to propose a relationship between the type of VA chosen for HD, and the risk of thrombogenesis. (J Vasc Access 2008; 9: 248-53)

Key words: Fibrinolytic activity, Chronic renal failure, Vascular access, D-dimers

INTRODUCTION

A successful hemodialysis (HD) procedure requires a functional vascular access (VA). Unfortunately, no major advances in the field of HD VA have been observed for the past 30 yrs, and this has probably contributed to VA dysfunction as being one of the most important causes of morbidity in the HD population (1). Access-related problems are responsible for 50% of the hospitalizations of HD patients (2). Currently, there are three main forms of HD VA: arteriovenous fistula (AVF), polytetrafluoroethylene

(PTFE) graft, and central venous catheter (CVC). Each of these forms has its own specific problems. AVF is the first choice for VA in patients undergoing chronic HD due to its relative low risk of infection and thrombosis. However, this type of VA has two major complications: initial failure to mature (primary non-function) and a later venous stenosis followed by thrombosis (3). A PTFE graft is relatively easy to place and ready to use; however, extremely high rates of stenosis, thrombosis, and infection are reported for this VA (4). CVC is the least desirable method of HD access. In a sense, every CVC placement repre-

sents a failure: failure to prepare a native AVF in advance of initiating dialysis; failure to detect failing AV access and maintain its function, or create a suitable alternative. Nevertheless, catheters are an unavoidable necessity for many patients who do not have functional AV access for any reason. In recent years, the use of CVCs has increased in both acute and chronic uremic patients, and this could underlie the increased morbidity and mortality observed in these patients. The type of insertion and the management of a CVC can lead to several complications, namely infections and thrombosis (5).

An inflammatory stimulus triggers the synthesis of acute phase proteins, namely C-reactive protein (CRP), a prominent marker of inflammatory response in the general population and in chronic kidney disease (CKD) patients (6-8). CRP increases significantly in hemodialyzed patients, as compared with healthy controls (8, 9) and this rise appears to be a common feature in hemodialyzed patients. Chronic inflammation is also associated with atherosclerotic cardiovascular disease (CVD). CRP has been proposed as a new risk marker for CVD events; the high rate of morbidity and mortality observed in aemodialyzed patients (10, 11) could reflect the overlapping of these proinflammatory conditions and/or the inflammatory process in CKD patients favoring CVD events.

CKD patients undergoing HD have been also associated with complex hemostatic disorders, clinically expressed with both bleeding tendency and hypercoagulability, though its etiology is poorly understood. A disturbance in fibrinolysis was reported in CKD patients; however, studies of different fibrinolysis parameters in regularly dialyzed patients have yielded conflicting results, with some indicating suppressed fibrinolysis and others showing increased fibrinolysis (12). Several hemostatic proteins are also considered endothelial cell function markers, namely plasminogen activator inhibitor 1 (PAI-1) and tissue plasminogen activator (tPA), being both secreted by endothelial cells.

There are a considerable number of studies regarding the association of the type of VA with inflammatory markers and fibrinolytic/endothelial cell dysfunction (13, 14). However, only a few have investigated simultaneously inflammatory and fibrinolytic/endothelial cell dysfunction markers.

This work aimed to study the association between fibrinolytic/endothelial cell function and inflammatory markers in CKD patients undergoing HD and recombinant human erythropoietin (rhEPO) therapies, and its relationship with the type of VA used for the HD procedure.

MATERIALS AND METHODS

Subjects

We studied 50 CKD patients undergoing regular HD (32 males, 18 females; mean age 64.5 ± 15.4 yrs) and rhEPO therapy. These patients were recruited, after giving informed consent, from two HD clinics during a 1-year period. In 11 patients, the VA used for HD was a tunneled CVC, and in 39 patients it was an AVF. The CVC were used for a median period of 36 months (12-84 months). The incidence of VA infection in the unit is 5.6%.

CKD patients were undergoing therapeutic HD three times per week, for 3-5 hr, for a median period of 36 months. All patients used the high-flux polysulfone FX-class dialyzers of Fresenius, 25 with FX60, 23 with FX80 and 2 with the FX100 dialyzer type.

Patients with autoimmune disease, malignancy, hematological disorders, and acute or chronic infection were excluded. We also excluded patients that presented, at the time of analysis with a positive blood culture result.

The control group included 25 healthy volunteers (8 males, 17 females; mean age 47.81 ± 14.69 yrs) presenting normal hematological and biochemical values, with no history of renal or inflammatory diseases, and, as far as possible, age and gender matched with CKD patients.

Assays

Blood samples were drawn from fasting controls and in the case of the hemodialyzed patients before the second dialysis session of the week. Blood samples were collected with and without anticoagulant [ethylenediamine tetraacetic acid (EDTA)], in order to obtain whole blood, plasma and serum.

Hemoglobin (Hb) concentration and white blood cell (WBC) count were measured using an automatic counter (Sysmex K1000, Hamburg, Germany); differential leukocyte counts were evaluated in Wright-stained blood films. Serum CRP was measured by immunoturbidimetry (CRP latex HS Roche kit, Roche Diagnostics) and an enzyme-linked immunoabsorbent assay was used for measurement of serum soluble interleukin 2 receptor (s-IL2R) (human IL-2 SRa, R&D systems, MN, USA). Serum albumin levels were measured using a colorimetric assay end-point method (Albumin Plus; Roche GmbH, Mannheim, Germany). Serum IL-6 levels were quantified using the BD™ Cytometric Bead Array Human Th1/Th2 Cytokine Kit II (BD Biosciences, USA, San Diego), and analyzed using the BD™ CBA software. Plasma levels of PAI-1, tPA and D-dimers were

evaluated by enzyme-linked immunoabsorbent assays (TintElize PAI-1, TintElize tPA and TintElize D-dimer, Biopool-Trinity Biotech Company, respectively). Serum iron concentration was determined using a colorimetric method (Iron, Randox Laboratories Ltd, Northern Ireland, UK), whereas serum ferritin and serum transferrin were measured by immunoturbidimetry (Ferritin, Laboratories Ltd, Northern Ireland, UK; Transferrin, Laboratories Ltd, Northern Ireland, UK). Transferrin saturation (TS) was calculated by the formula: $TS (\%) = 70.9 \times \text{serum iron concentration in } (\mu\text{g/dL}) / \text{serum transferrin concentration in } (\text{mg/dL})$.

Data analysis

For statistical analysis, we used the Statistical Package for Social Sciences, version 14.0. Kolmogorov Smirnov statistics were used to evaluate sample normality distribution. Comparisons between groups were performed using the Kruskal-Wallis test and the Mann-Whitney U test (data with a non-Gaussian distribution) or one-way ANOVA supplemented with Tukey's HSD *post-hoc* test (data with a Gaussian distribution). Spearman's rank correlation coefficient was used to evaluate relationships between sets of data. Adjustment of statistical differences for confounding factors (comorbidity, age, sex and body mass index) was performed by analysis of covariance, after log transformation of variavel (when nec-

essary). Multiple regression analysis using the stepwise method was used to determine independent factors affecting D-dimers levels. Significance was accepted at $p < 0.05$.

RESULTS

Tables I and II show rhEPO doses, hematological data, fibrinolytic and inflammatory cell markers, dialysis adequacy parameters, and nutritional and iron status, for controls and CKD patients.

Compared to controls, CKD patients presented with significantly higher counts of WBC and neutrophils, and higher levels of ferritin, CRP, s-IL2R, IL-6 and D-dimers. CKD patients also presented with significantly lower levels of Hb and PAI-1. The tPA/PAI-1 ratio was significantly higher for CKD patients.

When comparing the two groups of CKD patients, we found that those with a CVC VA presented with statistically significant lower levels of Hb, TS and albumin, and higher levels of neutrophils, CRP, IL-6, D-dimers and tPA. Statistical significance remained after adjustment for the confounding factors. A trend to higher rhEPO doses to achieve target Hb levels (11-12 g/dL) was found in CKD patients using a CVC as VA (Tab. I).

We also found in CKD patients, statistically significant correlations between D-dimer levels and inflammatory markers: CRP, albumin, s-IL2R and IL-6 (Fig. 1).

TABLE I - HEMATOLOGICAL DATA, FIBRINOLYTIC AND INFLAMMATORY CELL MARKERS, DIALYSIS ADEQUACY PARAMETERS, AND NUTRITIONAL AND IRON STATUS IN CONTROLS AND CKD PATIENTS

	Healthy Controls (n=25)	All CKD Patients (n=50)
Weekly rhEPO (IU/Kg) dose	–	271.29 (79.22-577.29)
Hb (g/dL)	14.12 ± 1.27	11.06 ± 1.77 a)
WBC (x 10 ⁹ /L)	5.80 ± 1.60	10.03 ± 4.54 a)
Neutrophils (x 10 ⁹ /L)	3.00 ± 1.00	6.08 ± 3.01 a)
Platelets (x 10 ⁹ /L)	242.12 ± 78.57	212.48 ± 98.39
CRP (mg/dL)	1.75 (0.76-4.70)	5.75 (1.90-14.00) a)
s-IL2R (nmol/L)	758.83 ± 234.95	4199.94 ± 1762.82 a)
IL-6 (pg/mL)	1.90 (0-3.75)	7.8 (3.85-15.05) a)
Albumin (g/dl)	–	3.84 ± 0.42
D-dimer (ng/mL)	63.50 (51.94-77.41)	157.26 (97.72-417.09) a)
tPA (ng/mL)	4.72 ± 2.95	4.60 ± 3.57
PAI-1 (ng/mL)	58.17 ± 24.25	42.85 ± 23.53 a)
tPA/PAI-1 ratio	0.09 ± 0.06	0.14 ± 0.11 a)
Transferrin saturation (%)	21.83 ± 7.97	22.89 ± 11.06
Ferritin (ng/mL)	85.10 (37.88-123.95)	389.65 (186.58-604.90) a)
K _{tv}	–	1.59 ± 0.29
Urea reduction (%)	–	26.98 ± 8.53
Protein catabolic rate (g/Kg/day)	–	0.85 ± 0.04

*Results are presented as mean ± one standard deviation or as median values (inter-quartile range). a) $p < 0.05$, vs. controls

Fig. 1 - Correlation between D-dimer levels and inflammatory markers in CKD patients: CRP (A), albumin (B), s-IL2R (C) and IL-6 (D). Circles represent patients with an AVF and triangle patients with a CVC.

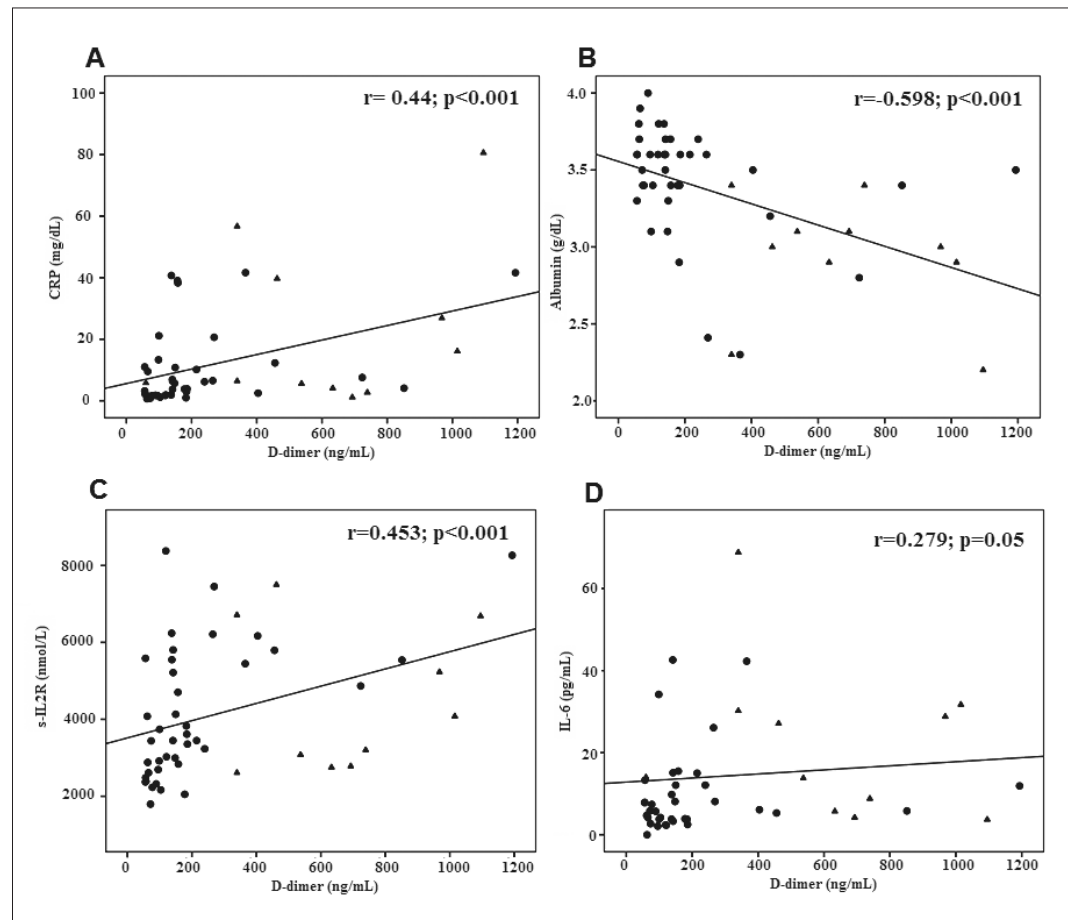


TABLE II - HEMATOLOGICAL DATA, FIBRINOLYTIC AND INFLAMMATORY CELL MARKERS, DIALYSIS ADEQUACY PARAMETERS, AND NUTRITIONAL AND IRON STATUS ACCORDING TO THE TYPE OF VASCULAR ACCESS

	CKD patients with AVF (n=39)	CKD patients with CVC (n=11)
Weekly rhEPO (IU/Kg) dose	156.01 (56.82-582.52)	456.12 (102.56-575.54)
Hb (g/dL)	11.27 ± 1.86	10.30 ± 1.18 a)
WBC (x 10 ⁹ /L)	10.11 ± 4.57	9.77 ± 4.64
Neutrophils (x 10 ⁹ /L)	5.90 ± 2.96	6.71 ± 3.27 a)
Platelets (x 10 ⁹ /L)	203.17 ± 85.19	245.45 ± 135.36
CRP (mg/dL)	4.10 (1.80-11.02)	6.46 (4.10-39.70) a)
s-IL2R (nmol/L)	4177.78 ± 1745.00	4278.51 ± 1909.72
IL-6 (pg/mL)	5.95 (3.80-12.40)	14.00 (5.70-30.20) a)
Albumin (g/dl)	3.94 ± 0.36	3.51 ± 0.46 a)
D-dimer (ng/mL)	141.63 (89.13-214.75)	632.88 (339.75-967.88) a)
tPA (ng/mL)	3.94 ± 2.21	6.99 ± 5.99 a)
PAI-1 (ng/mL)	40.74 ± 22.37	50.37 ± 27.06
tPA/PAI-1 ratio	0.14 ± 0.12	0.15 ± 0.08
Transferrin saturation (%)	25.19 ± 10.84	14.70 ± 7.63 a)
Ferritin (ng/mL)	454.66 (246.00-634.00)	374.00 (120.00-452.00)
K _{tv}	1.63 ± 0.27	1.50 ± 0.36
Urea reduction (%)	25.78 ± 7.01	31.12 ± 11.97
Protein catabolic rate (g/Kg/day)	0.87 ± 0.04	0.81 ± 0.03

*Results are presented as mean ± one standard deviation or as median values (inter-quartile range). a) p<0.05 vs. CKD patients with an AVF

Multiple regression analysis identified the weight ($\beta=-0.367$; $p=0.03$), neutrophil count ($\beta=0.399$; $p=0.001$) and s-IL2R ($\beta=0.279$; $p=0.022$) as the independent variables significantly associated with D-dimers ($R^2=0.427$).

DISCUSSION

CKD patients showed anemia, which, as reported in the literature (15), is mainly determined by the rate of renal insufficiency; and therefore, by the reduction in the renal secretion of erythropoietin. Additionally, CKD patients undergoing HD treatment presented with leukocytosis, neutrophilia and increased levels of IL-6, s-IL2R and CRP inflammatory molecules in the serum, confirming the presence of an inflammatory process.

In our CKD patients, we found low levels of PAI-1 and normal tPA plasma levels, suggesting that endothelial and fibrinolytic functions are not apparently altered in these patients (16). However, the tPA/PAI-1 ratio was increased in our CKD patients, and the increase in this ratio has been described as a novel risk marker for recurrent myocardial infarction (17). We also found an increase in D-dimers in our patients; however, it is uncertain if the higher fibrin fragment D-dimer value in CKD patients is a result of a higher activation of coagulation or of fibrinolysis. The higher tPA/PAI-1 ratio in CKD patients undergoing HD could suggest a higher fibrinolysis in these patients because only PAI-1 presented a significantly lower value. However, we did not measure fibrinolytic activity. On the other hand, an interesting cross-talk has been proposed to exist between inflammation and coagulation (18). The higher inflammatory status observed in CKD patients could actually underlie coagulation activation. For instance, CRP is known to promote tissue factor synthesis (19), a potent procoagulant, by monocytes. The correlations and the multiple regression analysis that we found between D-dimer levels and inflammatory markers, underlie this hypothesis. From that perspective, the higher D-dimer levels in the CKD patients can also be observed as a marker of the inflammatory process in these patients.

When comparing our results to that previously described, we found some conflicting results, namely for PAI-1 that was been described to be increased (20, 21) or normal (22). For tPA, we found descriptions of normal values, as we found in this work, and other describing higher levels (20). These differences could be due to the difference between the studied CKD patient population and due to the type of kit used in the laboratorial evaluation. Increase D-

dimer and tPA/PAI-1 ratio is the most consistent finding in most of the studies.

When we analyzed the results of the studied parameters according to the type of VA used, the CVC patients showed lower albumin and Hb values, and higher tPA, D-dimers, CRP and IL-6 levels as compared to those with an AVF, suggesting that these patients have an even higher inflammatory process. Our results also showed in CKD patients using a CVC as VA that they seem to require a higher rhEPO dose to achieve the target Hb levels, suggesting also cross-talk of erythropoiesis with inflammation.

It is important to note that we measured tPA antigen, which reflects both active free tPA and inert tPA bound to inhibitor(s) (23). Therefore, higher tPA values are not synonymous with higher activated fibrinolysis; higher t-PA values could result from endothelial dysfunction (23). Although tPA was higher in CVC patients, the tPA/PAI-1 ratio remained unchanged suggesting a similar activation of coagulation and fibrinolysis. The higher D-dimer values are more likely to result from enhanced coagulation probably linked to a higher inflammatory stimulus, than to a higher fibrinolysis, as only a trend to higher PAI-1 levels was observed in CVC patients.

In summary, our results showed a relationship between hemostasis and increased inflammatory markers in CKD patients. The increased levels of D-dimer, tPA and inflammatory markers in CKD patients using a CVC, led us to propose a relationship between the type of VA chosen for HD, and the risk of thrombogenesis. It seems reasonable to assume that these patients could present with an increased risk for CVD events.

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