Effects of acetate-free haemodiafiltration (HDF) with endogenous reinfusion (HFR) on cardiac troponin levels

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Abstract

Background. Haemofiltrate reinfusion (HFR) is a form of haemodiafiltration (HDF) in which replacement fluid is constituted by ultrafiltrate from the patient ‘regenerated’ through a cartridge containing hydrophobic styrene resin. Bicarbonate-based dialysis solutions (DS) used in routine haemodialysis and HDF contain small quantities of acetate (3–5 mMol/L) as stabilizing agent, one of the major causes of intradialytic hypotension. Acetate-free (AF) DS have recently been made available, substituting acetate with hydrochloric acid. Cardiac troponin (cTnT) constitutes an appreciable marker of myocardial damage and cardiac hypertrophy, and correlates with left ventricular mass.

Methods. The aim of this study was to assess the impact of the presence or lack of acetate in DS on cTnT levels in patients treated with HFR and to evaluate outcome of intra-session cardiovascular stability. Twenty-five patients devoid of major cardiovascular comorbidity were randomized and treated with AF HFR for 3 months. The same patients were subsequently treated by means of HFR with DS containing 3 mMol/L acetate for 3 months and finally with AF HFR for a further 3 months. Prior and subsequent to each treatment period, samples were collected for cTnT measurement.

Results. A significant decrease was observed in cTnT levels throughout the first session of AF HFR (1.32±0.35–1.12±0.31 ng/mL, P<0.05) with a subsequent rise being registered during HFR with acetate-containing DS (1.12±0.31–1.28±0.37 ng/mL, P<0.05) and a further drop from 1.28±0.37 to 1.21±0.35 ng/mL in the last
AF HFR period. During HFR with acetate-containing DS, a significant drop in systolic and diastolic arterial pressure was observed in conjunction with a higher heart rate at the end of the session.

**Conclusion.** We observed an increase in cTnT during HFR with acetate and drops manifested during HFR without acetate; it may therefore be concluded that the drop in cTnT level, significantly correlated with lack of acetate, is indicative of improvement of cardiac microvascular function.

**Keywords:** acetate-free dialysis solutions; cardiac troponin; dialysis solutions; haemodiafiltration; haemodiafiltration with endogenous reinfusion; HFR

**Introduction**

Cardiac troponin (cTnT) constitutes an appreciable marker of myocardial damage and cardiac hypertrophy, and correlates with left ventricular mass [1–4]. Evidence of an increased concentration of cTnT in dialysis patients with no signs of heart disease has been reported [5–7], and numerous studies have demonstrated the importance of this protein in acting as an independent marker capable of predicting cardiovascular events [4,8–13]. De Filippi et al. [3] detected high cTnT levels in 30–75% of patients, further maintaining that higher levels may be associated with an increased risk of coronary diseases. Lippi et al. [14] and Sommerer et al. [6] have underlined how variations in cTnT levels subsequent to dialysis may be associated with the type of membrane used. Moreover, the latter authors reported a significant correlation between cTnT levels and secondary arteriovenous fistulas and/or catheters likely produced by a chronic inflammatory condition frequently associated with this type of vascular access.

Over the years, considerable attention has been focused on the presence of acetate (3–5 mMol/L) as pH stabilizer in bicarbonate dialysis solution (DS), in view of its ability to produce negative reactions even when small quantities of the compound are employed. Pizzarelli et al. [15,16] compared the biological effects produced during on-line HDF with and without acetate in a cross-over study of 12 patients. On-line HDF was performed with reinfection in pre-dilution of 40 L of filtered dialysis solution containing 3 mMol/L of acetate and compared with the results obtained following administration of an acetate-free (AF) solution during sessions and 1 h after completion of sessions. HDF performed by means of acetate-containing DS and replacement fluids was seen to produce a 5- to 6-fold increase in acetate concentrations with respect to basal levels, displaying an acetate gain of ~75 mMol (+36% overall base gain). The toxic effects of acetate have already been widely acknowledged: induction of hypoxia, dyslipaemia and release of cytokines (IL-1, IL-6, TNF-α), activation of cyclooxygenase and lipoxygenase, and synthesis of thromboxanes and prostaglandins (TLB4 and PGE2). Amore et al. [17–19] demonstrated that acetate incubated with endothelial cells activates a massive release of nitric oxide (NO) through stimulation of the inducible synthesis of NO (iNOS). The large quantity of acetate-containing replacement fluids generated by DS may, in turn, give rise to a cascade of biochemical events leading to a potential long-term effect on cardiovascular morbidity and mortality of patients.

Noris et al. [20] compared the effects produced by dialysis performed with acetate (AHD), bicarbonate dialysis (BHD) and acetate-free biofiltration on NO synthesis and intradialytic hypotension. The data obtained significantly correlate with the possibility that cytokines and NO released in high quantities during AHD may contribute to intradialytic haemodynamic instability. Grandi et al. [21] subsequently confirmed this observation.

Selby et al. [22] conducted a clinical research on patients with BHD using an acetate-containing DS and on-line HDF with acetate-free DS (paired haemodiafiltration—PHF) with the aim of assessing whether the latter method was capable of eliminating both increase in cTnT levels (54 patients) and systemic haemodynamic instability (12 patients). Arterial pressure was lower during PHF, although no signs of increased cardiovascular instability or heart rate variations were observed. Both stroke volume and cardiac output progressively reduced during both treatments, although to a lesser extent during PHF than BHD (P=0.003 and P=0.0001, respectively) with a greater increase in peripheral resistance in BHD (P=0.0001). Prior to treatment, cTnT levels were lower in PHF than in BHD (P=0.023), subsequently displaying a decrease following PHF and, conversely, a rise after BHD (P=0.0001). The authors concluded that acetate-free PHF is correlated with a lower degree of deterioration of systemic haemodynamics, improved stability of arterial pressure and reduced suppression of myocardial contractility.

Tomo et al. [23] conducted a cross-over study on 24 patients treated with HDF in pre-dilution with acetate for 3 months followed by acetate-free HDF for a further 3 months. In acetate-free HDF, a significant reduction in C-reactive protein (CRP), interleukin-6 (IL-6) and pentosidine levels was demonstrated, while fetuin-A was significantly increased; the authors concluded that the use of acetate-free solutions improves the predictive value of cardiovascular risk variables.

**Haemodiafiltration with endogenous reinfusion**

HFR is a form of HDF that utilizes separated convection, diffusion and adsorption (Figure 1). A two-stage filter is applied that consists of a high-flux polyethersulfone filter in the first convective stage and a low-flux polyethersulfone filter in the second diffusive stage to enhance complete separation of convection from diffusion. In the convective phase of the first stage, pure ultrafiltrate (plasmatic water) passes through a sorbent cartridge containing 40 mL of hydrophobic styrene resin (Selecta Plus®, Bellco Srl, Miranda, Italy) constituted by numerous pores and channels that add to its extensive surface area (∼700 m²/g). Moreover, this process is devoid of problems related to coagulation and haemocompatibility due to the absence of red blood cells, inflammatory cells and platelets. Treatment is...
performed on a Formula Plus™ monitor (Bellco Srl) equipped with a particular software programme that automatically determines the best ultrafiltration flow rate (Quf).

The sorbent cartridge has a high affinity for several uraemic toxins and middle molecules including β2-microglobulin, homocysteine, angioegenin, leptin, parathyroid hormone (PTH), and several chemokines and cytokines [24–27]. Urea, creatinine, uric acid, Na⁺, K⁺, Ca²⁺, phosphate and bicarbonate are not adsorbed and remain unchanged after passage through the cartridge. These can be managed by diffusion during the second stage of filter. Thus, the ‘regenerated’ ultrafiltrate is an endogenous ultrapure replacement fluid with a physiologic content of bicarbonate.

**Aim of the study.** The aim of the present study was to assess the potential correlation between HFR with and without acetate on cTnT serum levels and intradialytic cardiovascular stability. The behaviour of anaemia was the secondary outcome of the study.

**Materials and methods**

The study was approved by the ethics committee, and informed consent was obtained from all study participants. A total of 656 patients attending 14 participating centres were evaluated, and 25 patients without any evident inflammatory, tumoral or metabolic disorders (21 males and four females—age 66.9±12.7 years, dialytic age 59.2±32.1 months, dry weight 64.6±9.7 kg) enrolled in the study. The causes underlying terminal renal failure included nephrosclerosis (13), diabetic nephropathy (two), glomerulonephritis (four), polycystic kidney (two) and undetermined nature (four).

Criteria applied for inclusion in the study were age ≥18 years, dialytic age >6 months, treatment three times weekly, treatment duration ~240 min and protein catabolic rate (PCR) >1.0. Patients affected by acute or chronic infections, malignant tumours, active systemic diseases, uncompensated hepatopathies, malnutrition, poorly functioning vascular access or with recirculation >10%, Kt/V <1.1, residual diuresis >100 mL/day, unstable diabetes mellitus, ischaemic or congestive cardiopathy (NY Heart Association class IV), cardiac pacemakers, clinically evident peripheral vasculopathy, poorly controlled hypertension, life expectancy <12 months or undergoing immunodepressive treatment or chemotherapy were excluded from the study.

**Definition of hypotension episode**

A hypotension episode was defined as a symptomatic fall of systolic blood pressure ≤80 mmHg requiring saline or plasma expander infusion.

**Study design**

The 1-year observational study was divided into four phases: the first featuring use of BHD with bicarbonate/acetate DS followed by three distinct three-monthly periods using HFR—acetate-free HFR 1 (AF HFR 1), standard HFR (Std HFR) and acetate-free HFR 2 (AF HFR 2) (Figure 2).

Throughout all HFR phases, identical parameters were applied: Bellco SG 40 filter [0.7 m² high-flux polysulfone (PS) in the convective stage and 1.7 m² low-flux PS in the diffusive stage], Selecta Plus™ sorbent cartridge (Bellco Srl), ultrafiltration rate (Quf) and reinfusion flow (Qinr) ~3 L/h, DS flow (Qd)=500 mL/min, dialysate temperature=37°C, blood flow (Qb)=300-350 mL/min and session duration=3.5–4 h. DS composition in BHD and Std HFR (mMol/L) was as follows: Na⁺ 138–140, K⁺ 1.5–3, Ca²⁺ 1.5, Mg²⁺ 0.5, bicarbonate 32, acetate 3, CI⁻ 109.5 and glucose 5.55. DS composition in HFR AF 1 and HFR AF 2

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**Fig. 1.** HFR outline.

**Fig. 2.** Study design (n=25). Each patient served as his or her own control.
Correlations among variables were assessed by multiple regression analysis, taking into account the whole period of study. Considerable variations were revealed for the following operating parameters: blood urea nitrogen (BUN), creatinine, uric acid, phosphates, iPTH, pH, HCO$_3^-$, total protein, albumin, C3, C4, total HDL and LDL cholesterol, triglycerides, leptin, Hb, iron, ferritin, transferrin, TSAT, cTnT (ELISA; DRG Instruments GmbH, Germany), IL-6, IL-10 and CRP.

Haematology tests were performed monthly at the beginning of the mid-week session for both BHD and HFR to monitor the following parameters: blood urea nitrogen (BUN), creatinine, uric acid, Kt/V, PCR, β2-microglobulin, Na, K, total calcium, phosphates, nALP, iPTH, pH, HCO$_3^-$, total protein, albumin, C3, C4, total HDL and LDL cholesterol, triglycerides, lecithin, Hb, iron, ferritin, transferrin, TSAT, cTnT (ELISA; DRG Instruments GmbH, Germany), IL-6, IL-10 and CRP.

Arterial blood pressure and heart rate were monitored at the beginning and end of each session, and all episodes of intradialytic hypotension were recorded. Pharmacological therapy was carefully monitored and dosages of the following measured: phosphate binders, vitamin D and/or derivatives, statins, ESAs and iron supplementation.

Statistical analysis

Each patient served as his or her own control. A descriptive analysis of the results obtained was performed by calculating mean values±SD. Data collected at the end of each three-monthly period were compared with those obtained at the end of the previous period using the t-test for paired data with Bonferroni’s correction for multiple comparisons (BHD end vs BHD start, AF HFR 1 end vs BHD end, Std HFR end vs HFR AF 1 end and HFR AF 2 end vs Std HFR end, respectively). A P-value of 0.05 was considered significant. Correlations among variables were assessed by multiple regression analysis, taking into account the whole period of study. Statistical evaluation was performed by means of the NCSS97 software package (NCS; Kaysville, UT, USA).

Results

Throughout the duration of the study, no significant variations were revealed for the following operating parameters: treatment time (229.0±23 min), Qb (316±21.2 mL/min), Qd (500 mL/min) and DS temperature (36.7±0.6°C). During the three HFR phases, the Quf and the Qinf were maintained at ~12 L/session.

No significant differences were observed between average values of BHD period vs HFR periods in BUN, creatinine, uric acid, plasmatic Na, K, total calcium, phosphates, nALP, iPTH, pH, HCO$_3^-$, total protein, albumin, C3 and C4, total HDL and LDL cholesterol, triglycerides and leptin. A slight, although significant, decrease was observed for Kt/V in HFR AF 1 vs BHD (P=0.036), whilst no significant variations were detected for PCR, dry weight and dialytic weight loss. A marked, but not significant, decrease in plasma levels of β2-microglobulin was observed in the shift from BHD to HFR periods (from 28.6±6.6 to HFR 24.7±8.1 mg/L). No significant variations were recorded throughout the study for phosphate binders and vitamin D and its derivates. Statin administration remained constant.

Table 1 illustrates the trend displayed by cTnT (Figure 3), systolic and diastolic blood pressure and heart rate at start and at end of the session, the number of hypotensive episodes per session and the quantity of normal saline infused during sessions (expressed in millilitres per session per month). Throughout the three HFR phases, no significant differences were observed in the number of patients taking antihypertensive drugs (HFR AF 1=16/25, Std HFR=18/25, HFR AF 2=19/25). However, a statistically significant decrease in cTnT levels was registered during the first HFR AF 1 (P<0.05), with a subsequent increase during Std HFR (P<0.05) and a new decrease during the last HFR AF 2. A drop in systolic blood pressure at the beginning of the session was recorded in the BHD period (P=0.036). At the end of the session, systolic blood pressure was significantly lower only in the Std HFR period (P=0.01). Modest but significantly lower values were recorded for diastolic blood pressure at the end of the session in the Std HFR period.
A considerable increase was registered in heart rate at the end of the session in the Std HFR period (P=0.001). During the BHD period, 68% of patients were taking at least one antihypertensive drug, during the HFR AF 1 period, 48%, in the Std HFR period, 56% and during the HFR AF 2 period, 56% (P=ns). No significant variations were recorded for quantity of normal saline administration. Hypotensive episodes were registered in 7.5% of BHD sessions, in 2.7% of Std HFR sessions and in 2.5% of HFR AF 1 sessions, in 3.1% of HFR AF 2 sessions. A significant inter-session decrease in systolic blood pressure was recorded only during the Std HFR session (P=0.004).

Table 2 shows values obtained for Hb, ESAs requirements (IU/month), serum iron, iron supplementation, ferritin, transferrin and TSAT. An increase in Hb (but non-significant) was observed during HFR AF 1 and continued to increase for the entire duration of the study. A significant decrease was recorded in ESAs requirements throughout the course of HFR periods, possibly due in part to an increased, although not significant, administration of iron i.v. Conversely, a significant ferritin increase (P=0.02) was manifested during HFR 1 and Std HFR AF, with transferrin concentration significantly decreasing (P<0.05) during HFR AF 2.

**Discussion**

The behaviour displayed by cTnT is of particular interest: it maintains stable levels during BHD (DS with acetate), drops significantly during HFR AF 1 (DS acetate free), rises equally significantly during the subsequent 3 months of Std HFR (DS with acetate) (Table 1, Figure 3) and then drops again during the HFR AF 2 period (DS acetate free). This finding confirms the observations reported from Selby et al. [22] maintaining that cTnT elicits potentially harmful cardiac effects triggered by the presence of acetate in DS.

The data obtained in the present study confirm the negative effect produced by acetate on peripheral vascular resistances. Accordingly, as can be deduced from Table 1, the presence of acetate in DS is correlated with higher instability of blood pressure, manifesting a drop in systolic arterial pressure in Std HFR. Otherwise, the outcome of diastolic arterial pressure at the end of the session would also be significantly lowered during the same period with a parallel increase in heart rate, thereby confirming the negative effects of acetate on cardiac inotropy and on peripheral vascular resistances, followed by a clear attempt at sympathetnic compensation. The results obtained appear to suggest that not only is the presence or absence of acetate capable of influencing hypotensive episodes but also that the method used may play an important role; indeed, compared with BHD, during all HFR periods a significant decrease in hypotensive episodes was registered. Despite the substitution of traditional acetic acid with HCl in DS, no significant variations were observed in acid-base balance throughout the entire course of the study. The findings obtained in this study revealed that a decrease in cTnT levels was closely correlated to the presence or lack of acetate. In addition, closed-circuit reinfusion of patient’s plasmatic water during HFR provided an absolute guarantee of sterility and apyrogenicity, and contributed towards reducing the effects of microinflammatory processes.

With regard to Hb, ESAs requirements, iron supplementation, serum iron, ferritin, transferrin and TSAT (Table 2), it should be noted that after the three initial study periods, during which a constant TSAT <20% was recorded, we deemed it opportune to implement intravenous administration of a non-significant higher dose of iron. On obtaining higher levels of ferritin and TSAT, iron dosage was reduced between Std HFR and the last HFR AF 2 phases so as not to overshoot the Hb target.

Nevertheless, the data obtained here demonstrate how HFR produces an improvement in erythropoiesis irrespective of the acetate content of DS. Indeed, an increase in Hb was recorded in the presence of an appreciable and signifi-
cant decrease in ESAs requirements. The increase in Hb was manifested in the first 3 months of HFR AF, displaying an additional modest progression over the subsequent 6 months. The decrease in ESAs requirements was recorded at a later stage once data concerning the increase in Hb had been acquired (Table 2).

Further studies should however be undertaken to confirm the direct correlation obtained between cTnT and acetate, using a larger study sample and prolonged observation period in an attempt to achieve statistically significant data.

List of participating centres:

ASL Cagliari, Quartu Sant’Elena (S. Murtas)
Policlinico Sant’Elena, Quartu Sant’Elena (M. Mascia)
Ospedale SS. Annunziata, Sassari (M. Cossu)
Ospedale SS. Trinità, ASL Cagliari (R. Ferrara)
Ospedale S. Martino, Oristano (G. Cogoni)
Ospedale S. Francesco, Nuoro (F. Cadini)
Ospedale Civile, Alghero (D. Casu)
Ospedale N.S. della Mercede, Lanusei (M. Passaghe)
Poliambulatorio Specialistico Distrettuale, Macomer (T. Ghisu)
Ospedale A. Segni, Ozieri (M. Ganadu)
Ospedale S. Camillo, Sorgono (F. Logias)

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Conflict of interest statement. P.M.G. is an external medical–scientific consultant of Bellco Srl; L.C. is an employee of Bellco Srl (Scientific Affairs).

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