The effect of on-line haemodiafiltration, vegetarian diet and urine volume on advanced glycosylation end products (AGEs), measured by changes in skin auto-fluorescence.

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Abstract

Background

Increasing dialyzer urea clearance has not improved patient survival. Haemodiafiltration (HDF) has been reported to reduce cardiovascular mortality. HDF increases middle sized solute clearances. Advanced glycosylation end products (AGEs) are associated with increased cardiovascular mortality. We wished to determine whether HDF reduces AGEs.

Methods

Skin auto-fluorescence (SAF) measures AGEs deposited in the skin. We compared SAF measurements 12 months apart in high flux hemodialysis (HD) and HDF patients.

Results

At enrollment SAF was not different (HD 3.34±0.71 vs HDF 3.48±1.05 AU). At 7th months, one haemodiafiltration centre reverted to haemodialysis, and one haemodialysis centre converted to haemodiafiltration. SAF increased (3.36±0.71 to 3.82±0.88 AU, $p<0.001$), in the 66 patients treated solely by high flux HD,

whereas there was no change for 47 exclusively treated by HDF (3.45±1.13 to 3.44±0.85 AU, p>0.9), however SAF increased in 34 patients switching from HDF to high flux HD (3.52 ±0.94 vs 3.88±1.05, p<0.05), with no significant change for 33 patients converting from high flux HD to HDF (3.32±0.72 to 3.48±1.07 AU, p>0.3). On multivariate analysis, SAF was associated with older age (β coefficient 0.013, p=0.002), prescription of insulin (β 0.29, p=0.016), lanthanum (β 0.36, p=0.004), and warfarin (β 0.62, p=0.012). Whereas vegetarian diet and > 250 mL/day residual urine volume were negatively associated with SAF, (β -0.58, P=0.002 and β -0.26, P=0.033 respectively).

Conclusion

Haemodiafiltration prevented an increase in SAF, whereas SAF increased over time with high flux haemodialysis. In addition, residual urine output and vegetarian diet were associated with lower AGE deposition.

Introduction

Despite advances in dialysis technology the 5-year survival of haemodialysis (HD) patients remains below that of some of the more common solid organ malignancies (1). Cardiovascular disease remains the major cause of death in HD patients, and in addition to the traditional risk factors recognized in the general population, there are additional risk factors for the HD patient, including uraemic toxins. Advanced glycation end products (AGEs), can be classified as low molecular weight (<12 kDa) or high molecular weight (>12 kDa), with low molecular weight AGEs typically being free proteins or peptide-bound proteins, whereas high molecular weight tend to be protein-bound molecules. Serum AGEs accumulate in patients with chronic kidney disease, due to a combination of reduced renal clearance and increased production. AGEs are produced from the reaction of amino acids and sugars by the Maillard reaction. AGEs are reactive molecules, which increase oxidative reactions causing inflammation and promoting vascular damage, fibrosis and accelerating atherogenesis (2).

Haemodialysis effectively clears small water-soluble molecules by diffusion and studies have reported a small reduction in circulating low molecular weight AGEs post dialysis (3). Another study from the same group reported that there was a difference in the reduction in plasma levels when using low flux compared to high flux dialyzers(4). Haemodiafiltration by adding convection, increases middle molecule clearance, and studies of patients treated by haemodiafiltration have reported a reduction in all cause and cardiovascular mortality with greater convective clearance compared to standard dialysis(5). As haemodiafiltration may be expected to increase plasma AGE clearance, we wished to determine whether haemodiafiltration reduced AGEs.

Plasma AGE concentrations are known to fluctuate pre and post dialysis treatments and also with dietary intake of preformed AGEs (6).
As increased circulating AGEs lead to tissue deposition, so tissue deposits more reliably reflect longer term exposure. AGEs deposited in subcutaneous tissues

can be detected using auto-fluorescence (SAF) techniques, and tissue concentrations are not affected by dialysis treatments(4), or dietary calorie or protein intake (7), however, the glucose free dialysate might reduce SAF(8). Previous studies have reported that increasing SAF is associated with increased mortality and cardiovascular mortality in dialysis patients (9-11). We therefore wished to determine whether treatment with haemodiafiltration reduced tissue AGE deposition compared to high flux haemodialysis.

Materials and Methods

We conducted a prospective observational study in 180 chronic haemodialysis patients dialyzing in four centres under the care of a tertiary university centre, measuring SAF 12 months apart. At the start of the study two of the dialysis centres provided haemodiafiltration, and two haemodialysis. During the 12- month period one haemodiafiltration centre returned to haemodialysis and one haemodialysis centre converted from haemodialysis to haemodiafiltration at 7th months after recruitment period (Figure 1). The inclusion criteria are all patients who receiving chronic haemodialysis 3 times a week and consented to participate this study. All patients with chronic kidney failure stage 5 are dialysed in satellite dialysis centres under the care of the Royal Free hospital according to accommodation address, irrespective of medical conditions or co-morbidity. The baseline characteristics, co-morbidity monthly blood chemistry and urine output was collected from hospital electronic medical records. Smoking history, and dietary intake were obtained by direct interview with the patients. We used the Stoke-Davies patient co-morbidity grading system (12). All patients were dialysed using the same type of high flux polysulfone dialysers (Fresenius FX series, Fresenius Medical, Bad Homberg, Germany), anticoagulated with low molecular weight heparin (13, 14) and containing glucose 1g/L in dialysate. The median dialysis session duration was 4 hours and patients attended for thrice weekly treatments to achieve a minimum single pool Kt/V target of 1.2 per session, and median post dilution haemodiafiltration infusion volume of 15 L/session. Exclusion criteria included inability to provide informed consent, patients who declined to participate in the study or those in whom SAF AGEs could not be measured due to excessive skin pigmentation.

SAF was measured using an AGE reader (DiagnOptics, Groningen, Netherlands), which directs ultraviolet light (UV), intensity range 300-420 nm (peak 370 nm), through the skin for excitation AGEs deposited in subcutaneous tissues, and then measures auto-fluorescence light with a spectrophotometer with a range 300-600 nm (15). The AGE reader is fitted with an additional light source for those with darker skin pigmentation. SAF was measured three times on the volar surface of the non-fistula arm, adjusted for skin colour (calculated by AGE reader software), and the average value recorded.

Ethics

Patients provided informed consent, and this observational study was registered with national health service (NHS) ethics (IRAS129559) and approved by London Camden and Islington research ethics committee (13/LO/0912) and registered (ISRCTN70556765).

Statistical Analysis

Data was checked for normality, and paired samples, and medications for individual patients were compared with paired student's T-test for numerical data and McNemar's test for categorical data and Wilcoxon paired test for non-parametric data. Univariate and multivariate analysis was tested using a mix linear model (repeated measures ANOVA). The variables that had a P value <0.1 were included in the multivariate model. We used STATA version 12 (Stata Corp LLC, Texas, USA) for all analyses. Results are expressed as variable, mean ±standard deviation, median (interquartile range) or percentage. A p value of <0.05 was considered clinically significant.

Results

A total of 550 chronic haemodialysis patients were potentially eligible for study, 218 patients were excluded with 8 patients unable to provide informed consent, 10 patients declined to be studied and despite an additional light source SAF could not be measured in 200 patients due to dark skin pigmentation (figure1). A total of 332 patients dialyzing in the 4 centres had initial SAF measurements. Patient demographics and past medical history, results are set out in table 1 according to dialysis modality at the time of first measurement. At enrollment, 2 dialysis centres exclusively provided haemodiafiltration and 2 centres exclusively haemodialysis. Patient demographics are set out in table 1.

After 12- month follow-up 54 patients had died, 40 patients transferred to other centres, 57 patients underwent kidney transplantation, and 1 patient recovered renal function (Figure1). We were therefore only able to repeat SAF measurements in 180 patients after 12 months. The mean SAF increased from 3.41±0.88 to 3.67±0.96, p<0.001. At enrollment, there were no differences in SAF between those patients treated by haemodiafiltration compared to those by haemodialysis, although the haemodiafiltration cohort had been treated by dialysis for a longer time, fewer patients treated by haemodiafiltration had residual renal function, and higher baseline cardiovascular co-morbidity (Table 2). Serum β2 microglobulin at study entry was similar in the haemodialysis and haemodialfiltration groups. After follow-up for 30 months, mortality was higher in those patients who had higher initial SAF of ≥3.625 AU (27.5 vs 16.8%, p=0.04) independent of dialysis modality.

During the study period, 7th months after initial SAF measurements were made, one haemodiafiltration centre reverted to haemodialysis, and one haemodialysis centre converted to haemodiafiltration. Thus, there were 47 patients who continued haemodiafiltration in one dialysis centre, 34 patients reverted from haemodiafiltration to haemodialysis in a second dialysis centre, 33 patients converting from haemodialysis to haemodiafiltration in a third dialysis centre and 66 patients continuing with high flux haemodialysis in the fourth dialysis centre (figure1). There was a significant increase in SAF in those patients who continued with high flux haemodialysis throughout the 12 months (3.36±0.71 to 3.82±0.88 AU, P<0.001), whereas there was no change in those continuing with haemodiafiltration (3.45±1.13 to 3.44±0.85 AU, p=0.91). SAF also increased significantly in those reverting from haemodiafiltration to high flux haemodialysis (3.52±0.94 to 3.88±1.05, p=0.032), whereas the increase in SAF for those converting from haemodialysis to haemodiafiltration was much less (3.32±0.72 to 3.48±1.07, P=0.31), and not significant (figure 2). The mean change of SAF during 12 months was significantly different for those treated

exclusively by haemodiafiltration ((-0.014 ± 0.12 AU), compared to those reverting from haemodiafiltration to haemodialysis (0.37±0.19 AU), converting from haemodialysis to haemodiafiltration (0.18±0.18 AU) and those treated exclusively by haemodialysis (0.48±0.16), p=0.021.
Whereas mean serum β2 microglobulin increased in those treated exclusively by haemodialysis (29.2 ±8.2 to 30.3 ±7.0 mg/L), β2 microglobulin decreased in the exclusively haemodiafiltration group (32.9 ±7.9 to 26.3 ±6.3 mg/L). Interestingly, the haemodiafiltration is not showing significantly associate with AGEs deposition in the univariate as well as multivariate mix linear model (table 3), it is probably due to the small sample size in each subgroup and carryover effect of haemodialysis during study period.

SAF was greater both at enrollment in Caucasians compared to non-Caucasians (3.53±0.87 vs 3.11±0.86 AU), and after 12 months (3.84 ±0.95 vs 3.25±0.84), p<0.001. Similarly, SAF was lower for those eating an exclusively vegetarian diet both initially (2.86 ± 0.56 vs 3.46 ±0.89 AU, p=0.009) and on follow-up (2.94 ±0.49 vs 3.74±0.96 AU, p=0.001).

We then investigated which factors which were associated with a change in SAF. An higher SAF was associated with increasing age, white ethnicity, diabetes, lower residual renal function and prescription of clopidogrel (used after coronary artery stenting), lanthanum carbonate and warfarin, whereas both a vegetarian diet and higher serum albumin were associated with lower SAF (table 3). Serum phosphate was greater in those prescribed lanthanum carbonate (1.65±0.46 vs 1.45±0.46 mmol/L, p<0.05).

Using a multivariable mixed linear model, we found that older age, prescription of insulin, lanthanum carbonate and warfarin were independently associated with a significant increase in SAF accumulation, whereas vegetarian diet and residual urine volume >250 ml/day significantly attenuated SAF deposition (Table 3).

Discussion

At the start of study, we found no difference in SAF and B2 microglobulin between those treated by high flux haemodialysis compared to haemodiafiltration. However, the haemodiafiltration group had been treated by dialysis for longer and had less residual renal function, and greater previous cardiovascular comorbidity, all of which are recognized to be associated with higher SAF, as such these confounders most likely reduced any potential benefit from haemodiafiltration on reducing serum AGEs and increasing β2 microglobulin clearance at study baseline (16).

On follow-up after 12 months, whereas there was a modest, non-significant fall in SAF in the haemodiafiltration group, there was a significant increase in SAF in the haemodialysis cohort. Ramsauer et al, reported reduce SAF and plasma AGEs with glucose free dialysate(8). We used glucose at 1 g/L in the dialysate, so in theory the haemodiafiltration group would have had a potentially greater glucose exposure during treatment, but despite this those treated exclusively by high flux haemodialysis had greater increase in SAF. In addition, whereas SAF increased significantly in those who switched back from haemodiafiltration to haemodialysis, SAF only marginally and insignificantly increased in those who converted from haemodialysis to haemodiafiltration.

This would suggest that although the clearance of AGEs during a single haemodiafiltration session may be marginal compared to lengthening the session time (6), in the longer term haemodiafiltration treatments of similar session length of high flux haemodialysis potentially reduce the deposition of tissue AGEs(17). We did not measure serum AGEs, which previous study showed more reduction with low flux dialysis compared to high flux dialysis potentially due to back filtration during high flux dialysis(4). Moreover, mean serum β2 microglobulin increased in the haemodialysis group, whereas the mean fell in the haemodiafiltration group, the supposition would be that haemodiafiltration could reduce circulating small and middle-sized AGEs. However, the increase SAF accumulation more pronounce in inclusive high flux haemodialysis group, the high flux haemodialysis not associate with SAF accumulation in mix linear model. This is probably due to the small sample size in each group as well as the carry over effect of high flux haemodialysis during study period. Last but not least, the accumulation of SAF in exclusively haemodialysis group is significant higher when compare to exclusively haemodiafiltration (figure 2). Whether the reduced cardiovascular mortality recently reported with higher volume haemodiafiltration(18) , is due to reduced AGE deposition remains speculative and would require further studies.

Previous studies have reported increased SAF with white ethnicity (9), chronological age, and diabetes (10), however, another study in dialysis patients have reported an decrease SAF in diabetes (19). This may be due to the spectrum of patients meeting the world health organization definition of diabetes, ranging from diet control through to insulin requirement, and that glycated haemoglobin may be similar for those prescribed oral medications and insulin. As such, on multivariate analysis we found the association was with insulin prescription rather than glycated haemoglobin or whether a diagnosis of diabetes had been made. We found an association with prescription of warfarin and an increase in SAF. Only a small minority of patients were prescribed warfarin, so potentially introducing statistical bias, but in our centre warfarin was prescribed for metal heart valve replacements, and in patients with atrial fibrillation and either dilated left atrium or reduced left ventricular ejection fraction < 30%, both of which are associated with increased AGEs. In addition, warfarin inhibits the activity of serine proteinases, some of which regulate soft tissue and vascular calcification. This action of warfarin may potentially increase AGE tissue accumulation. Heart failure is associated with increased inflammation, and AGEs are increased with myocardial infarction(20), so the SAF accumulation might be increased by chronic inflammation. In our centre lanthanum carbonate is prescribed in cases of patients with increased serum phosphate concentrations in whom a calcium based binder is contra-indicated. Although we cannot exclude an effect of lanthanum per se (21), it is more likely that lanthanum was prescribed to patients with greater phosphate retention, and more severe hyperparathyroidism, and possibly with greater soft tissue calcification(22).

Residual renal function, as expected was associated with a reduction in SAF (19), and dialysis patient survival is greater for those who retain some residual renal function(23). As haemodialysis sessions result in a temporary reduction in residual renal function, there has been increased interest in incremental approaches to starting patients on dialysis(24), and approaches to preserve residual renal function(25).

We also found that patients eating a vegetarian diet had lower SAF, and vegetarian diet was associated with lower SAF over time. Patients eating vegetarian diets have been reported to have lower SAF (26), and also lower circulating protein bound azotaemic toxins(27). Western diets are generally rich in meat and processed food that might contain high levels of preformed AGEs compared with the vegetarian diet. A low AGEs diet may not only lower circulating AGEs and thus lower SAF deposition, but additionally alteration the gastrointestinal biome, as alter the production of other azotaemic toxins (28).

The limitations of our study include the observational nature and as such we can only report associations and not causality. We were unable to measure SAF in 36% of our patient population. Although the AGE Reader is fitted with an additional light source, as with previous studies we were unable to measure SAF in the majority of our black African and Afro-Caribbean patients. (reference Chaudhri S, Fan S, Davenport A Pitfalls in the measurement of skin autofluorescence to determine tissue advanced glycosylation content in haemodialysis patients. Nephrol. 2013;18(10):671-5). In addition, we did not measure SAF at the time two of the dialysis centres switched dialysis modality. Not all dialysis patients were included in the study, and a number of patients could not have repeated measurements due to transplantation, moving to different dialysis centres and mortality. As such we cannot exclude residual confounding due to patient selection and survivor bias. However, we were able to study a substantial number of patients.

In our prospective study, SAF increased over time with high flux haemodialysis, whereas, SAF did not increase in those patients treated exclusively with haemodiafiltration. In other patient groups, increasing SAF is associated with increased cardiovascular mortality(29). Whether this effect of haemodiafiltration in attenuating the accumulation of SAF compared to haemodialysis can potentially explain the reports of reduced cardiovascular mortality recently reported with haemodiafiltration treatments(5), remains to be determined. However, haemodiafiltration can only clear the fraction of circulating low molecular weight AGEs, and is unable to clear the larger molecular weight AGEs, and as such other extracorporeal treatments would be required to effectively remove all circulating AGEs and other protein bound azotaemic toxins (30). As such, our study also highlights the importance of residual urine output in dialysis patients to increase the clearance of circulating AGEs to prevent tissue deposition and also the importance of differences in diet which may reduce the ingestion of pre-formed AGEs.

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Table 1. Baseline characteristic of haemodialysis patients at enrollment. Data expressed as mean ±SD, or median (interquartile range) or percentage (%). Ethnicity: Caucasian (C), Asian (A), African-Afro-Carribean (AAC).

Parameters	Values
Age, years (mean±SD)	66.7±13.9
Male gender (%)	65.6
Ethnicity C/A/AAC (%)	71.1/18.9/10
BMI (kg/M²)	26.7±5.7
Dialysis vintage (months)	41 (17-81)
Vegetarian (%)	8.9
Smoker (%)	37.2
Diabetes (%)	54
Hypertension (%)	64.4
History of CVD (%)	33.3
History of PVD (%)	13.9
Stoke -Davies Co-morbidity score	
0 (%)	22.2
1 (%)	52.2
2 (%)	25.5
Urine volume (mL/day)(Median, IQR)	0 (0-652)
Urine volume ≥ 250 mL/day (%)	36 (20)
Hb(g/dl)	11±1.4
Serum albumin (g/L)	39.5±4.3
HBA1C (%)	6.6±1.9
B2microglobulin(mcg/mL)	29.4±16.2
CRP(mg/L), (Median, IQR)	
KT/V	1.66±0.37

Table 2. Baseline characteristics of patients regarding to two modes of haemodialysis; haemodiafiltration (HDF) and high flux haemodialysis (HD). Data expressed as mean ±SD, or median (interquartile range) or percentage (%). Skin auto-fluorescence advanced glycosylation end-products (AGEs) in arbitrary units (AU), Ethnicity – Caucasoid (C), Asian (As), African-Afro Caribbean (Af), dialysis adequacy (Kt/Vurea), haemoglobin (Hb), C reactive protein (CRP), glycated haemoglobin (HbA1c), cardiovascular disease (CVD), peripheral vascular disease (PVD), angiotensin converting enzyme inhibitor (ACEI), angiotensin receptor blocker (ARB).

Parameter	high flux HD (n=99)	HDF(n=81)	P value
SAF (AU)	3.34 ± 0.71	3.48 ± 1.05	0.297
Age (year)	65.3 ± 12.97	68.4± 14.77	0.14

Ethnicity C/As/Af	68/21/10	60/13/8	0.67
Post dialysis weight(kg)	72.6±18.53	73.7±15.66	0.68
KT/Vurea	1.67±0.37	1.65±0.37	0.71
Body mass index kg/m²	26.7±5.6	26.7±5.7	0.96
Dialysis vintage(month), (Median, IQR)	26(57)	57(70)	0.008
Hb(g/dl)	10.9±1.6	11.1±1.1	0.223
β2microglobulin(mcg/mL)	28.5±8.2	31.0±24.6	0.46
PTH (pg/mL), (Median, IQR)	36.4(51)	28.1(35)	0.39
Vitamin 25-OHD3 (nmol/L)	45.5±32.8	45±39.9	0.96
CRP(mg/L), (Median, IQR)	7(11)	6(10)	0.28
Serum calcium (mg/dL)	9.0±0.8	10.0±0.8	0.24
Serum phosphate (mmol/L)	1.5±0.5	1.5±0.4	0.88
Serum albumin (g/L)	39.1±4.2	39.9±4.4	0.17
Serum cholesterol (mmol/L)	3.87±1.1	3.9±1.1	0.83
HBA1C (%)	6.66±2	6.67±1.8	0.97
Urine volume (Median, IQR)	441(1108)	0(195)	0.001
Vegetarian (%)	7 (7)	9(11)	0.34
Smoker (%)	38(38)	29(36)	0.72
Stoke-Davies score (%)			0.649
0	21(21)	19(23)	0.72
1	50(51)	44(54)	0.61
2	28(28)	18 (23)	0.82
Male (%)	63(63)	55(68)	0.55
Diabetes(%)	46(46)	35(43)	0.66
History of CVD (%)	25(25)	35(43)	0.01
History of PVD(%)	14(14)	11(14)	0.91
B blocker (%)	28(28)	24(34)	0.84
ACEi(%)	17(17)	9(11)	0.25
ARB(%)	12(12)	4(5)	0.09
Statin(%)	60(61)	57(70)	0.17
Insulin (%)	27(27)	15(19)	0.17
Calcium based phosphate binder (%)	48(48)	48(59)	0.15
Sevelamer(%)	17(17)	21(26)	0.15
Alphacalcidol(%)	81(82)	73(90)	0.12
Aspirin (%)	58(59)	51(63)	0.55
Clopidogrel (%)	10(10)	10(12)	0.63
Lanthanum (%)	13(13)	12(15)	0.75
Cinacalcet(%)	8(8)	6(7)	0.87
Warfarin (%)	4(4)	4(5)	0.77

Table 3. Univariate and multivariate model of factors associated with skin auto-fluorescence accumulation during study period. Ethnicity – Caucasoid (C), Asian (As), African-Afro Caribbean (Af), peripheral vascular disease (PVD), glycated haemoglobin (HbA1c),

Parameters	B coefficient	Confidence interval	P value	B coefficient	Confidence interval	P value
Chronological age	0.01	0.002 to 0.02	0.018	0.013	0.005 to 0.02	0.002
Male gender	-0.2	-0.45 to 0.46	0.111	NS	NS	NS
Race C/As/Af	-0.419	-0.7 to -0.14	0.003	NS	NS	NS
Body mass index	0.009	-0.1 to 0.03	0.372	NS	NS	NS
Diabetes	2.75	0.04 to 5.1	0.022	NS	NS	NS
History of PVD	0.31	-0.03 to 0.65	0.073	NS	NS	NS
Vitamin D (25-OHD_3)	0.004	-0.00003 to 0.007	0.052	NS	NS	NS
Haemodiafiltration	-0.11	-0.29 to 0.6	0.198	NS	NS	NS
C reactive protein	0.002	-0.0001 to 0.005	0.062	NS	NS	NS
Serum albumin	-0.022	-0.44 to -0.0001	0.049	NS	NS	NS
HbA1C	0.005	0.0016 to 0.009	0.005	NS	NS	NS
Clopidogrel	0.29	0.006 to 0.58	0.046	NS	NS	NS
Insulin	0.21	-0.42 to 0.46	0.1	0.29	0.05 to 0.52	0.016
lanthanum	0.37	0.11 to 0.63	0.006	0.36	0.11 to 0.61	0.004
Cinacalcet	0.13	-0.15 to 0.41	0.37	NS	NS	NS
Warfarin	0.6	0.08 to 1.13	0.024	0.62	0.14 to 1.1	0.012
Vegetarian Diet	-0.7	-1.11 to -0.29	0.001	-0.58	-0.96 to -0.21	0.002
Urine volume > 250 ml/d	-0.29	-0.55 to -0.24	0.032	-0.26	-0.49 to -0.21	0.033

Figure 1. Study Consort Flow chart

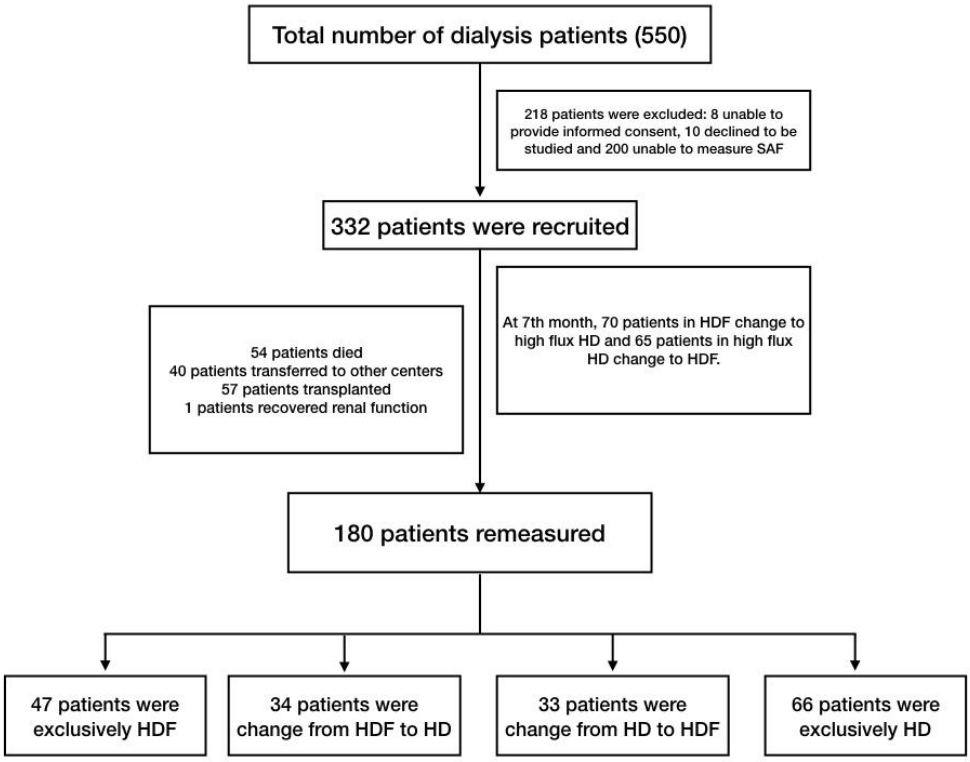
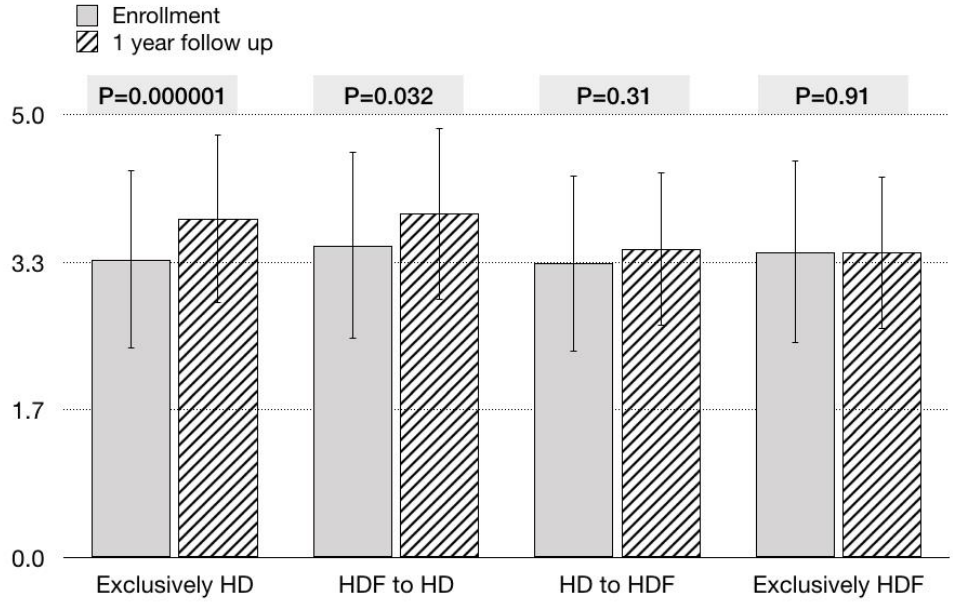


Figure 2. Skin auto-fluorescence measurements (SAK) in arbitrary units (Au) measured at study enrollment and then after 12 months in patients treated by exclusively by high flux haemodialysis (HD) or haemodiafiltration (HDF), and in the two dialysis centres which changed modes from high flux haemodialysis to haemodiafiltration and from haemodialysis to haemodiafiltration, respectively.



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