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Nontraditional cardiovascular risk factors in end-stage renal disease : studies on inflammatory markers and thyroid hormones

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Chapter 4

Variations in C-reactive protein during a single hemodialysis session do not associate with mortality

4

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Abstract

Background: An increase in C-reactive protein (CRP) levels during a single hemodialysis (HD) session has been associated with mortality. These associations, however, are difficult to understand from the current understanding of CRP metabolism.

Methods: In 190 Swedish HD patients from the Mapping of Inflammatory Markers in Chronic Kidney Disease (MIMICK) cohort, CRP was measured before and after a HD session. During follow up, events of death and censoring were recorded and hazard ratios were calculated and analyzed as a function of CRP variation. Results were replicated in 94 Dutch HD patients from the Netherlands Cooperative Study on the Adequacy of Dialysis (NECOSAD). In this cohort, also correlation and kappa statistics were calculated to assess concordance in CRP changes amidst multiple dialysis sessions from the same individuals.

Results: In both cohorts, mean CRP values did not increase during a single HD session. In the MIMICK, median (IQR) dialysis vintage was 29.0 (14.8-57.0) months. In both crude (HR [95% CI]: 1.008 [0.971 to 1.047]) and multivariate Cox models (0.996 [0.949 to 1.046]), no association was observed with mortality. In the NECOSAD, individuals endured 6.0 (6.0 to 12.0) months on dialysis. No association was found with mortality neither in a crude (0.961 [0.908 to 1.018]) nor in an adjusted analyses (0.978 [0.923 to 1.037]). Finally, the concordance between changes in different sessions was poor.

Conclusions: CRP changes during a single HD session do not associate with mortality, thereby adding to the biological uncertainty concerning the ability of CRP to rise in such short period.

Introduction

Patients with chronic kidney disease (*CKD*), and especially those with end stage renal disease (*ESRD*), are at considerable increased risk of premature death.¹ Since the surplus in *CKD* mortality is strongly associated with a state of persistent inflammation and chronic activation of the acute phase response, the identification of factors involved in the pathogenesis of the inflammatory response is of considerable therapeutic interest.²⁻⁵

In addition to *CKD* related factors, such as decreasing renal function, comorbidities, infections or the uremic environment per se,⁶ the hemodialysis (*HD*) procedure has been suggested to play a pivotal role in the development of inflammation. Indeed, several studies have shown that intradialytic activation is associated with increased fractional synthesis rates of albumin and fibrinogen as well as of pro-inflammatory cytokines, leading to a state of increased muscle-protein catabolism.⁷⁻¹⁰ While the long-term consequences of intradialytic activation (that is, increased inflammation) on the muscle may, indeed increase the mortality risk,^{11,12} it is uncertain whether these inflammatory fluctuations are as such valid prognosticators of the patient's outcome. A previous study based on the *NECOSAD* cohort reported that an increase in C-reactive protein (*CRP*) during a single *HD* session was positively related to subsequent mortality.¹³ However, while *CRP* is predominantly produced in the liver as induced by Interleukin-6 (*IL-6*) and typically raises after 6-8 hours from a major tissue damage,¹⁴ this somewhat surprising finding is difficult to understand from the current biological perspective. Since external validation of observational findings in unrelated populations is a fundamental step in the assessment of purported risk factors, the predictive power for death of intradialytic *CRP* changes has still to be confirmed in other cohorts of dialysis patients. This confirmation is of particular relevance because *CRP* levels appear highly variable across different studies in *ESRD* patient.¹⁵⁻¹⁷ With this background in mind, we used available data from two historical cohorts of dialysis patients to investigate and further clarify the association between changes of plasma *CRP* levels during a single *HD* session and subsequent outcome.

Subjects and methods

Subjects

This study comprises individuals from two independent patient cohorts. The first one corresponds to the Mapping of Inflammatory Markers in Chronic Kidney Disease (*MIMICK*) cohort, the protocol of which has been described elsewhere in more detail.¹⁸ This cohort includes adult patients (n=224) on maintenance *HD* therapy recruited during the period of October 2003 to September 2004 in six dialysis units in the Stockholm-Uppsala (Sweden) region. Out of these individuals, 190 patients had *CRP* and body weight measurements performed both before and after the index session. A comparison between the included individuals and the overall cohort revealed no differences in general characteristics (data not shown). The study Ethics Committee of Karolinska Institutet, Stockholm, Sweden approved the study protocol and informed consent was obtained from each

patient. From inclusion on forward, events of death were recorded, with no loss to follow up. The second cohort is a selection from the Netherlands Cooperative Study on the Adequacy of Dialysis (*NECOSAD*) study, described in more detail elsewhere.¹⁹ This is a prospective follow up study in which all new ESRD patients from 38 dialysis centers in the Netherlands were asked to participate between 1997 and 2002. Patients were re-assessed every six months. During follow up, events of death and censoring, due to renal transplantation, leaving the study or the end of follow up, were prospectively recorded. For the current analysis, individuals were selected conditionally on having completed at least a second HD session six months after an initial observation with an identical protocol. Ninety-six patients met these criteria and again, no major differences were observed with regards to general baseline characteristics when compared with the overall cohort (data not shown). Of these ninety-six individuals, an overlap of eleven patients existed with the sample reported on by Korevaar et al.¹³ The Medical Ethics Committees of all participating Dutch centers approved the protocol and informed consent was obtained from every individual.

For the MIMICK cohort, plasma CRP levels were measured before and after a single dialysis session (index session). The same measurements were recorded in the *NECOSAD* individuals, which additionally collected data on one or two additional consecutive dialysis sessions (six months apart as per protocol). Co-morbidity was classified according to Davies et al.,²⁰ on a seven point scale which was later on simplified into three risk categories (low, medium and high co-morbidity risk). Nutritional status was measured by means of the subjective global assessment which was trans-calculated to a three-point scale.²¹ Weight was recorded before and after each dialysis and rounded up to the nearest decimal. Body Mass Index (BMI) was calculated as the body weight (kg) divided by the squared height (m).

Biochemical Methods

In both cohorts, venous blood was obtained immediately before and after the dialysis session. In the MIMICK cohort, samples were stored at -80°C for a period ranging between 2 to 14 months before high-sensitivity CRP measurements were performed. High-sensitivity CRP concentration was measured by commercial immunometric assays (LKCRP, DPC, Siemens, California) on an Immulite Analyzer (Immulite, DPC, Siemens, California), which had a detection limit 0.1 mg/L. Coefficient of variation for a 20-fold repetitive measurement of a single sample is reported of 7.5%. In the *NECOSAD* cohort, samples were stored at -80 °C degrees for a period ranging from 4 to 8 years before high-sensitivity CRP measurements were performed. We recently reported that this different storage time did not affect the measurements.²² Measurements were done by means of particle-enhanced immunonephelometry using a standard CardioPhase high-sensitivity CRP for BNII (Dade Behring Holding GmbH, Liederbach, Germany) which had a detection limit 0.1 mg/L. Coefficient of variation for a 20-fold repetitive measurement of a single sample is reported of 7%.²² While in the MIMICK cohort serum albumin levels were measured with bromecresol purple, immunonephelometry was used in the *NECOSAD*.

Statistical Methods

For both the MIMICK as well as the NECOSAD cohort, a change in serum CRP levels over a HD session was calculated as the levels after- minus before- dialysis in which an increase is reflected by a positive value. Body weight change was calculated as the weight before- minus the weight after- dialysis. In addition, relative body weight change (Relative Δ body weight) was calculated by dividing the change during dialysis by the weight before dialysis and multiplying this value by 100 to obtain percentages. To take into account potential volume shifts during the index session (hemoconcentration), CRP levels after dialysis were adjusted by means of the formula proposed by Bergström & Welhe²³ based on bodyweight change. As a sensitivity analysis in the NECOSAD, correction for hemoconcentration was also done using the albumin ratio during HD (described in more detail by Korevaar et al.¹³). Because this approach did not yield any differences in findings, results are not presented. To assess whether the mean change of serum CRP levels (both unadjusted and adjusted for hemoconcentration) deviated from zero (no change), one sample t-test statistics were calculated.

In each cohort, individuals demonstrating an increase in serum CRP levels during the HD session (>0 mg/L) were grouped as “increase” group, while individuals showing either no-change in CRP or a decrease (≤ 0 mg/L) were classified as “no-increase” group. In the comparison of baseline characteristics between both groups in each cohort, data is presented as means plus standard deviations (SD) and medians plus interquartile ranges (*IQR*), depending on the variable distribution and parametric and non-parametric tests were applied as appropriate. In the analyses of survival in MIMICK subjects, the Cox proportional hazards model was used to calculate hazard ratios for a change in CRP over the index session. Since age, gender, comorbidity, nutritional status, and dialysis vintage are known to influence CRP levels and mortality, they were included as confounders in a multivariate Cox model. Moreover, to take into account the potential confounding effect of CRP levels before dialysis, the logarithmic transformed value was included in the crude as well as in the multivariate analysis, together with the relative body weight change. The proportional hazards assumption was tested by calculating the correlation between the Schoenfeld residuals of each covariate and the survival rank for each patient. For all variables, this test was non-significant, indicating no violation of the proportional hazards assumption.

To assess the concordance in changes of CRP between subsequent hemodialysis sessions in NECOSAD individuals, Spearman correlation coefficients and Cohens Kappas were calculated. Identical statistical methods were used for the survival analyses in NECOSAD patients. Since individuals in the NECOSAD sample were selected on having fulfilled measurements during at least two dialysis sessions, follow up started from the second session onwards. For the same reason, characteristics at the time of the second session were included as confounders in the multivariate Cox model. For all the statistical tests, SPSS version 17.0 (SPSS Inc., Chicago. IL. USA) was used. Furthermore, a 95% confidence interval (95%CI) not including 1 for all hazard ratios and a p-value < 0.05 for all other tests was considered to be statistically significant. Kaplan Meier curves were created using Prism 5.02 (Graphpad, 1992).

Table 1. Mean change of serum CRP levels during a single hemodialysis session, both crude and after correction for hemoconcentration

	Uncorrected	Corrected by albumin ¹	Corrected by body weight ²
<i>MIMICK, n=190</i>			
Δ CRP, mg/L [#]	1.99 (5.23) *	-	-0.76 (4.49)
Relative Δbody weight, % [#]	-3.5 (1.9) *		
<i>NECOSAD, n=94</i>			
Δ CRP 1 st HD session, mg/L [#]	1.03 (8.75)	-0.79 (7.78)	-1.83 (7.73)*
Relative Δbody weight 1 st HD, % [#]	-2.5 (1.6) *		
Δ CRP 2 nd HD session, mg/L [#]	1.17 (6.60)	0.41 (5.38)	-0.90 (5.57)
Relative Δbody weight 2 nd HD, % [#]	-2.7 (1.6) *		

1. Corrected by means of the albumin ratio. In 9/94 individuals, albumin was not measured.

2. Corrected by means of the formula suggested by Bergstrom and Welhe²¹ based on body weight change.

Means plus SD. Deviations from zero were tested with a one sample t-test, with a * denoting p<0.05.

Results

In a crude analysis, mean CRP levels were significantly increased after the HD session in the MIMICK cohort (**Table 1**), but not in the two NECOSAD recorded sessions. Correction of CRP levels after the HD session for hemoconcentration made these differences disappear.

In the MIMICK cohort, mean age (SD) was 62.6 (14.0) years and median (IQR) vintage on dialysis 29.0 (14.8-57.0) months. Furthermore, of all individuals, 56.3% were men and 26.3% had diabetes mellitus. As shown in **Table 2**, no substantial differences were observed between patients who increased or did not increase in their CRP levels during a HD session with regards to age, sex, dialysis vintage, time on dialysis per week, relative body weight change, co-morbidities and type of dialyzer used. However, BMI and body weights before- and after- dialysis were higher in the CRP increase group.

During a median follow up of 41.3 (IQR: 22.2 to 48.5) months, 87 out of 190 patients died, of which 58/125 (46.4%) and 29/65 (44.6%) in the no-increase and increase groups, respectively. As shown in the Kaplan Meier survival curve (**Figure 1**), no difference in survival was noted between the two groups. When a change of CRP during the index session was analyzed as a continuous variable in a univariate Cox proportional hazards model, no association was found with mortality neither in the crude analyses, (HR [95%CI]: 1.008 [0.971 to 1.047]), nor after adjustment for CRP levels before dialysis, age, sex, malnutrition (SGA score), Davies co-morbidity score, relative body weight change and dialysis vintage (0.996 [0.949 to 1.046]). In the NECOSAD cohort, individuals were on average 65.2 (13.7) years of age and endured a median (IQR) time of 6.0 (6.0 to 12.0) months on dialysis (**Table 3**). When the sample was restricted to changes during the first session, no substantial differences with respect to age, gender, co-morbidity, type of filter, BMI and relative body weight change were observed between the increase group and no-increase group. However, the increase in serum albumin levels was substantially higher in the increase group.

Table 2. Baseline characteristics of MIMICK patients according to the CRP change during a single HD session

	No increase group ($\Delta\text{CRP} \leq 0$ mg/L) n = 125	Increase group ($\Delta\text{CRP} > 0$ mg/L) n = 65	P value
Age, years [#]	62.3 (14.0)	63.2 (14.0)	0.6
Men, % [¶]	58.4	52.3	0.4
BMI, kg/m ² ^{1,#}	24.0 (5.4)	25.6 (5.0)	0.05
CRP before, mg/L [*]	6.3 (1.5 to 21.0)	7.6 (2.1 to 24.0)	0.7
CRP change, mg/L ^{2,#}	-2.1 (4.2)	1.9 (3.8)	-
Body weight before, kg [#]	72.5 (18.0)	77.4 (17.1)	0.07
Body weight change, kg ^{3,#}	-2.6 (1.3)	-2.7 (2.0)	0.8
Relative body weight change, % ^{4,#}	-3.6 (1.7)	-3.4 (2.3)	0.5
Dialysis vintage, months [*]	28.0 (12.5 to 57.0)	30.0 (15.0 to 57.0)	0.6
Time on dialysis per week, hours [*]	12.0 (12.0 to 13.5)	12.0 (12.0 to 13.5)	0.4
Type of dialyzer, Synth/cell. based, % [¶]	95.2/4.8	93.8/6.2	0.7
Part with Diabetes Mellitus, % [¶]	24.0	30.8	0.3
Comorbidity, low/middle/high, ^{5,¶}	24.8/52.8/22.4	15.4/52.3/32.3	0.2
Malnourished, % ^{1,6,¶}	47.2	53.1	0.9

1. In a few subjects, not all characteristics were measured.
 2. CRP changes were calculated as CRP after- minus before the index session, in which serum CRP concentrations after the index session were adjusted for volume shifts during dialysis by use of the formula suggested by Bergstrom and Welhe. ²³.
 3. Weight change was calculated as weight before dialysis minus weight after dialysis.
 4. Relative body weight change was calculated as body weight change divided by body weight before dialysis times hundred.
 5. Comorbidity was defined according to Davies (scale 1 to 3).
 6. Malnourishment was defined as a SGA score >2 on a scale of 1 to 3.
- [¶] Categorical variables were compared using a Chi square test.
- ^{*} Data are expressed as median (IQR) and differences were tested by means of a Mann Whitney U test.
- [#] Data are expressed as mean (SD) and difference were tested by means of an independent sample t-test.

In the NECOSAD cohort, when comparing changes amidst different sessions, a low concordance and agreement existed between the changes in the index session and the second session (n=94), as expressed by non-significant kappa and correlation statistics with a value close to zero (**Table 4**). Out of the 94 patients, having fulfilled measurements during the first and second measurement, 47 attended a third HD session in which CRP variation was assessed. Again, concordance and agreement between both sessions were very low (**Table 5**). During the median follow up of 18.4 (IQR: 9.3-41.6) months, 52 patients died. However, no differences were observed between the patients that showed or did not show an increase in their CRP value during the HD session (**Figure 2**). No association was observed between ΔCRP as a continuous variable with mortality in a crude Cox model (0.961 [0.908 to 1.018]) nor when adjusted for the abovementioned confounders (0.978 [0.923 to 1.037]).

Table 3. Baseline characteristics of NECOSAD patients according to the CRP change during a single HD session

	No increase group (Δ CRP \leq 0 mg/L) n = 61	Increase group (Δ CRP $>$ 0 mg/L) n = 33	P value
Age, years [#]	64.4 (14.3)	66.6 (12.5)	0.4
Men, % [¶]	49.2	63.6	0.2
BMI, kg/m ² ^{1,#}	25.0 (4.8)	26.1 (3.3)	0.2
CRP before, mg/L [*]	11.4 (3.0 to 21.3)	8.6 (3.1 to 21.5)	0.5
CRP change, mg/L ^{2,#}	-3.6 (8.9)	1.5 (2.3)	-
Albumin before, g/L [#]	38.7 (3.9)	39.0 (4.0)	0.7
Albumin change, g/L ^{3,#}	2.4 (4.3)	5.1 (4.7)	0.006
Body weight before, kg [#]	74.5 (13.5)	76.4 (12.8)	0.5
Body weight change, kg ^{4,#}	-1.8 (1.2)	-2.1 (1.0)	0.2
Relative body weight change, % ^{5,#}	-2.4 (1.7)	-2.6 (1.3)	0.6
Dialysis vintage, months [*]	6.0 (6.0 to 12.0)	6.0 (6.0 to 12.0)	0.7
Time on dialysis per week, hours [*]	10.5 (8.0 to 12.0)	12.0 (9.0 to 12.0)	0.2
Type of dialyzer, Synth/cell. based, % [¶]	86.0/14.0	92.7/6.3	0.5
Part with Diabetes Mellitus, % [¶]	9.8	6.1	0.1
Comorbidity, low/middle/high, % ^{6,¶}	39.3/52.5/8.2	36.4/57.6/6.1	0.9
Malnourished, % ^{1,7,¶}	32.7	27.3	0.7

1. In a few subjects, not all characteristics were measured.
 2. CRP changes were calculated as CRP after- minus before- the index session, in which serum CRP concentrations after the index session were adjusted for volume shifts during dialysis by use of the formula suggested by Bergström and Welhe.²³
 3. Albumin changes were calculated as Albumin after- minus before- the index session.
 4. Weight change was calculated as weight before dialysis minus weight after dialysis.
 5. Relative body weight change was calculated as body weight change divided by body weight before dialysis times hundred.
 6. Comorbidity was defined according to Davies (scale 1 to 3).
 7. Malnourishment was defined as a SGA score \geq 2 on a scale of 1 to 3.
- ¶ Categorical variables were compared using a Chi square test.
- * Data are expressed as median (IQR) and differences were tested by means of a Mann Whitney U test.
- # Data are expressed as mean (SD) and difference were tested by means of an independent t-test.

In a sensitivity analysis in the MIMICK cohort, only individuals with a vintage on dialysis under 15 months were included (n=44). Moreover, in additional analyses, cut-off values were chosen differently, testing the median CRP change, the 75th percentiles, or even including a third stable group (-0.5 to 0.5mg/L change) to the increase ($>$ 0.5 mg/L) and decrease ($<$ -0.5 mg/L change) Group. All these analyses did not yield any difference in findings. Because the previous study¹³ was performed with a non high-sensitivity CRP assay, we further excluded in the survival analyses of both cohorts all patients with a CRP concentration before, and/or after dialysis under 3 mg/L. Finally, we also re-ran the survival analyses without correction for hemoconcentration. Also in these analyses, findings did not change.

Table 4. Percentage of individuals with concordant/discordant CRP changes during the first and second HD sessions in the NECOSAD sample

n=94 Change during 1 st session	Change during 2 nd session	
	Increase	Decrease
Increase, n (%)	15 (16.0)	18 (19.1)
Decrease, n (%)	23 (24.5)	38 (40.4)

$\kappa = 0.075$, $p = 0.5$, Pearson correlation: -0.01 ($p = 0.9$)

Table 5. Percentage of individuals with concordant/discordant CRP changes during the second and third HD sessions in the NECOSAD sample

n=47 Change during 2 nd session	Change during 3 rd session	
	Increase	Decrease
Increase, n(%)	5 (10.6)	11 (23.4)
Decrease, n(%)	10 (21.3)	21 (44.7)

$\kappa = -0.01$, $p = 0.9$, Pearson correlation: 0.07 ($p = 0.6$)

Discussion

The current study demonstrated in two independent European cohorts that on average, CRP levels do not significantly change during a single HD session. In addition, we demonstrated a lack of association between these changes and mortality. Furthermore, it was observed that the concordance and correlation in CRP changes amidst different sessions was very poor.

In both cohorts, corrected CRP levels did not increase during dialysis. These findings are in disagreement with previous works in which serum CRP levels were found to be elevated after a HD session.^{8,24-26} In all these studies, however, CRP levels after dialysis were not adjusted for hemoconcentration, leading possibly to a misinterpretation of the results. Indeed, the significant crude CRP increase observed in the HD session of MIMICK patients was explained by the effect of hemoconcentration, as volume extraction (indicated by relative body weight change) was higher in this patient material. In a cross sectional study, Park et al.²⁶ found characteristics of left ventricle hypertrophy (LVH) to be more prevalent amongst individuals who showed an increase in CRP levels during dialysis (responders). However, as basal levels were substantially higher in responders and no correction for the effect of hemoconcentration on CRP measurements took place, it could be argued that the observed rise in serum CRP levels is, at least partly, secondary to the effect of fluid removal during dialysis. Moreover, ultrafiltration was slightly higher in the response group, thereby aggravating this effect. In this perspective, the association between an increase in CRP during dialysis and LVH could be considered as an invalid consequence of the known association between high CRP levels and LVH.²⁷

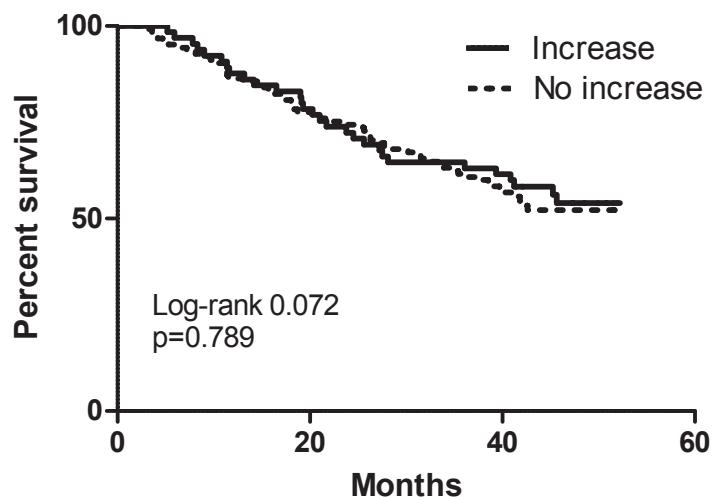


Figure 1. MIMICK sample: Survival plots by change of serum CRP during dialysis

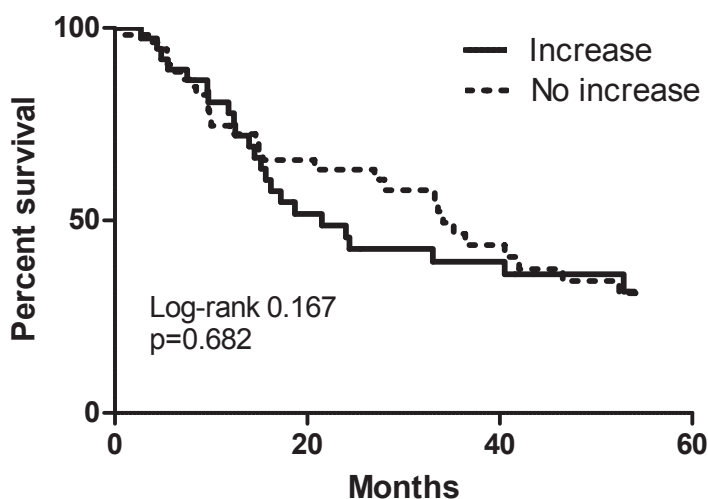


Figure. 2. NECOSAD sample: Survival plots by change of serum CRP during dialysis

Most importantly, the current study contradicts our previous finding in the NECOSAD cohort in which an increase of 1 mg/L in serum CRP levels during dialysis was associated with a statistically significant 8 percent higher mortality risk during follow up.¹³ There are some important similarities and differences between these two studies, which are of importance when analyzing the strengths and limitations of the current analysis. Firstly, whereas Korevaar et al.¹³ studied HD patients with a median vintage of 6.0 months on dialysis, the MIMICK cohort is composed of HD patients with a median vintage of 29.0 months on dialysis. Thus, it could be argued that a survival bias masked a potential association between a change in serum CRP levels and mortality in the MIMICK population. More specifically, when considering a pro-inflammatory response to extracorporeal circulation to be a subject-specific characteristic, individuals with a more pronounced inflammatory response to dialysis would have died before reaching 29 months on HD therapy. This reasoning is further supported by the fact that on average, age was lower and dialysis vintage approximately three fold higher in the MIMICK cohort as compared with the NECOSAD cohort. However, the theory of a possible survival bias is contradicted by the absence of an association between changes of CRP during dialysis and mortality in the current NECOSAD sample and in the sensitivity analysis of MIMICK patients, restricted to those being less than 15 months on dialysis.

Secondly, it could be argued that while CRP levels in our previous observation¹³ were measured using an assay with a detection limit of 3 mg/L, our current analysis utilized high-sensitivity CRP assays. Nonetheless, sensitivity analyses excluding CRP values <3 mg/L in the present analyses also showed no differences in outcome prognostication in both cohorts. Additional limitations of our study include the fact that the protocol was not specifically designed to evaluate changes during the HD session, and several factors that potentially could influence CRP generation or degradation during the dialysis session, such as fluid status, were not taken into account. Finally, it would have been desirable to measure CRP repeatedly after the HD session over a longer time window to study whether CRP rose afterwards.

When putting these results in the context of the current understanding of CRP biology, more uncertainties add to the previously observed association between an intradialytic CRP change and mortality. When the dynamics of the acute phase response was studied after the aggressive stimulus of cutting the sternal bone during open heart surgery, CRP levels rose after approximately 7 hours.^{14,28} Because a dialysis session typically lasts 4-5 hours, an increase of CRP within this period is biologically less plausible. On the other hand, while extrahepatic CRP production has been reported in adipocytes²⁹ and endothelial cells³⁰ in experimental conditions, we cannot exclude the possibility that specific phenotypes, for example obese individuals, or certain genetic profiles may translate, under the exposure to the uremic milieu,³¹ in a faster CRP production from alternative tissues in response to the intermittent HD stimulus. However, this hypothesis has to date no substantiation and it is unknown if, and to what extent other tissues may contribute to systemic CRP levels in the context of uremia. In the interpretation of the current results, we have assumed the established statement that the vast majority of circulating CRP comes from hepatic production.³² Also, as Kaysen et al.¹⁵ pointed out, the acute phase response generally spans multiple dialysis sessions, thereby suggesting the value of intradialytic causes to be of less importance. Moreover, intradialytic changes in inflammatory markers seem to be very complex and influenced by many factors, such as adhesion of molecules to the dialyzer membrane, shifts between extravascular and intravascular compartments, and activation of the inflammatory response by extracorporeal circulation.^{33,34}

As apparent by the lack of consistency amidst the different HD sessions in our study, CRP is probably not a valid marker to monitor the intradialytic inflammatory response. Focus should instead go out to more adequate markers. For example, in a study by Boehme et al.,³⁵ blood leaving the dialyzer produced more Pentraxin 3 (*PTX3*) than blood withdrawn before the dialysis session. In another study by Friedrich et al.³⁶ intracellular RNA levels in leucocytes encoding TNF-alpha, IL-1 beta and IL-8 increased significantly during dialysis. Since the intracellular compartment does not seem to be affected by volume extraction during dialysis, no differences are encountered when adjusting values after dialysis for hemoconcentration in this specific study.

Based on findings in the current study and the difficulty to explain an increase in CRP levels within the course of a single HD session from a biological perspective, we conclude that CRP changes during dialysis do not associate with mortality. However, whether patients exhibiting a more pronounced inflammatory response to extracorporeal circulation, have an increased mortality risk, is still of substantial interest. Therefore, future research should focus on understanding the consequences of activating other inflammatory markers or interleukins during HD.

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