Biochemistry and Biomarkers of Inflamed Patients: Why Look, What to Assess

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Specific laboratory tests and physical findings are available to the practicing clinician that should raise the suspicion of inflammation. Inflammation is related to specific clinical outcomes. Once identified, changes in clinical practice may affect the level of inflammation in individual and or groups of dialysis patients with the hope that these changes may in turn affect outcome in a positive manner. Standard clinical tests and observations associated with inflammation are hypoalbuminemia, erythropoietin resistance, decreased iron saturation accompanied by high ferritin, frailty, low serum creatinine, reduced total and LDL-cholesterol, and increased C reactive protein (CRP). Inflammation is strongly associated with loss of physical function, dyslipidemia (low LDL- and HDL-cholesterol, increased triglycerides), and anemia that is unresponsive to erythropoietin. Inflammation is associated with cardiovascular events, increased hospitalization, and death. Correctible causes of inflammation are tunneled dialysis catheters, arteriovenous grafts, catheter infection, periodontal disease, poor water quality, and dialyzer incompatibility. Obesity also is a source of cytokines but may be less amenable to treatment. Inflammation is multifactorial in dialysis patients. Some sources are recognizable and correctable, such as vascular access type, clinical infection, and water quality, and some are not. Inflammation is strongly associated with outcome.

Origin Prevalence and Consequences of Inflammation in the CKD Patient

Patients with chronic kidney disease (CKD) have an increased prevalence of inflammation as measured by any of a number of biomarkers; however, the direct relationship between inflammatory biomarkers such as IL-6 and GFR is weak (1). Among patients who enrolled in the Modification of Diet in Renal Disease (MDRD) study, there was no difference in age-adjusted C-reactive protein (CRP) levels compared with the National Health and Nutrition Examination Survey (NHANES; 1999 to 2000) population (2), whereas Eustace et al. (3) did find an association with the risk of having an increased CRP level with decreasing estimated GFR (eGFR) in the NHANES III population. Thus, CKD is likely associated with risk factors that favor the presence of inflammation rather than be a direct cause of inflammation. The biomarker that is most readily measurable by the practicing clinician to detect the presence of inflammation is CRP and is part of the innate immune system. In epidemiologic studies of patients without CKD, the upper tertile of CRP values consists of values >3 mg/L (4) and is strongly associated with cardiovascular risk. In contrast, the median CRP value for hemodialysis patients is approximately 8 mg/L (5). CRP values within a dialysis patient population are also quite variable temporally (6).

Because inflammation is highly prevalent among patients with CKD but is not always present, it is important to identify risk factors that might be causal. In some studies, serum levels of IL-6, IL-1, and TNFα were significantly increased in patients with renal failure, and no difference was observed between long-term and not yet dialyzed patients (7,8), suggesting that risks contributed by treatment do not contribute substantively to inflammation. To support this hypothesis, several factors have been proposed to promote CKD-associated inflammation, including increased oxidative stress (9–12) and the accumulation of postsynthetically modified proteins [advanced glycation end products (13,14) and products of carbonyl stress (15)], resulting from reduced renal clearance.

Several groups, however, have found that inflammation is increased primarily in dialyzed patients (16–18). Once renal replacement therapy is initiated, it is likely that treatment-associated risk such as vascular access choice contributes substantively to inflammation.

Sources of Inflammation

Vascular access type is associated with mortality in hemodialysis patients (19). Baseline albumin is correlated with the type of vascular access being used [arteriovenous (AV) fistulas > AV grafts > permanent catheters > temporary catheters] (20). Although this could represent selection bias, the observation that albumin decreases after insertion (21) and increases after catheters are removed (22) suggests otherwise.

Initial vascular access type is associated with the risk of sepsisemia (23), clearly a process associated with inflammation. Unrecognized infections (24) and biofilm deposited on plastic catheters (25,26), including peritoneal catheters (27) and periodontal disease (28,29), are all potential treatable sources of inflammation in dialysis patients.
Another potential source of inflammation unique to hemodialysis patients is that engendered by interaction of circulating monocytes with dialysis membranes or bacterial products, lipopolysaccharide, and bacterial DNA that may be present in dialysate (30), all of which are associated with inflammation as determined by increased CRP and IL-6 levels. Significant reduction in the use of erythropoietic stimulating agents (ESAs) and a decrease in both CRP and IL-6 have been observed after purification of the water supply (31,32) or upgrading the water system in a dialysis facility (33).

CRP levels have been observed to be higher in HD patients compared with patients on peritoneal dialysis (PD), both in small cross-sectional studies (34), as well as in longitudinal studies (35), although we did not note a difference when we compared CRP levels in patients in whom we have performed detailed analysis of the relationship between albumin homeostasis and inflammation (36). The rate of infectious complications seems to not be significantly different by modality (37), but the inflammatory response to infectious processes within the peritoneal cavity in comparison to bacteremia and sepsis may differ. Evaluation of the relationship between albumin levels in the two populations is confounded in part by the effect of transperitoneal albumin losses in PD, causing a lower than anticipated serum albumin concentration in PD patients (36,38).

Adipose tissue, especially visceral adiposity, is a source of cytokines and is associated with inflammation both in patients without renal failure (39) and among dialysis patients (40), although among dialysis patients, inflammation is expressed at greater levels at patients at both extremes of body mass (41), perhaps reflecting the effects of wasting resulting from inflammation among patients having lowest body mass index (BMI). Although loss of renal function per se may lead to oxidative stress and thus promote inflammation, the correlation between CRP or IL-6 and estimates of GFR does not approach significance (1), and initiation of dialysis does not reduce inflammation (42), suggesting that a cause other than retention of dialyzable solutes is responsible for inflammation. Infections are an obvious source of inflammation in dialysis patients. Infections represent the second leading cause of death and hospitalization among dialysis patients (43).

### Potential Injurious Effects of Inflammation

Hypoalbuminemia was noted to be a powerful risk factor for mortality among dialysis patients by Lowrie and Lew (44). Although hypoalbuminemia was presumed to result primarily from malnutrition, Yeun et al. (45) found that, although albumin predicted mortality in a cohort of hemodialysis patients, when CRP was placed in the regression model, it replaced albumin. Eighty percent of patients with a CRP level of greater than 11.5 μg/ml were dead within 28 mo. This value represented the upper quartile of their patient population. Zimmerman et al. (46) found that both CRP and albumin were independent predictors of all-cause mortality in HD patients. However, CRP contributed more to the risk of death and also replaced albumin as a risk factor when both were included in a multiple regression model. Bologa et al. (47) reported greater than 60% mortality in HD patients having IL-6 in the upper tertile within 26 mo.

Infectious events are associated with subsequent cardiovascular or cerebrovascular events in adults (48), with subsequent increases in carotid intimal media thickening in children (49), and are associated with an increased risk of cardiovascular events in dialysis patients (50). There are a variety of potential causal links in this chain. Fibrinogen is a positive acute phase protein and correlates with CRP. Fibrinogen is an independent cardiovascular risk factor (51). The lipoprotein Lp(a) is another powerful risk factor for vascular disease (52). Normally its plasma level is regulated in response to the size of isoform inherited; those with low molecular weight isoforms have high plasma levels and an increased risk of vascular disease and those with high molecular weight isoforms have low plasma levels and no increased risk. Inflammation causes increased levels of Lp(a) independent of isoform (53). Thus, individuals with the high molecular weight isoform may have plasma levels of this atherogenic lipoprotein increased. In inflamed individuals, HDL levels decrease (54), and the apo A-I that normally composes about one half of the proteins in HDL is replaced by serum amyloid A (SAA) (54). This form of HDL is chemoattractive to macrophages and the vascular endothelium and has reduced capacity to reduce oxidized LDL (54). LDL is also more likely to be oxidized both by the action of myeloperoxidase, a product of activated neutrophiles that chlorinates a tyrosine residue on apo B100, thus oxidizing it, and exposure to ceruloplasmin-bound copper (54). Ceruloplasmin is another acute phase protein and thus its serum concentration is also increased during the acute phase response. It should be noted that, in the study of Zimmerman et al. (46), inclusion of CRP in a multiple regression model removed SAA, Lp(a), and fibrinogen from the model. Taken in isolation, this analysis would suggest that other acute phase proteins are simply reporters of inflammation, whereas CRP plays a causative role in vascular injury. However, as will be discussed subsequently, CRP is unlikely to be on the causative pathway. The associations obtained from multiple regression models more likely exposes the weakness in this type of analysis in determining which proteins(s) are playing a role in initiating injury.

A small uncontrolled trial of short daily dialysis found that patients treated with short daily treatments exhibited a decline in left ventricular mass index accompanied by a decline in CRP and ESA resistance compared with controls (55). Whether inflammation and left ventricular hypertrophy (LVH) are both consequences of changes in arterial structure and function or whether inflammation actually plays a role in causing LVH either directly or by its effect on vascular resistance vessels is not clear at this time; however, aspects of cardiovascular disease beyond simply the interaction between inflammation and the vascular endothelium either directly or through the effects of inflammation on modulation of lipoprotein structure and function may be of importance.

As discussed above, CRP does not seem to be on the causal pathway linking inflammation and vascular disease. Patients having increased CRP levels as a consequence of gene polymorphism do not have the anticipated increase in cardiovascu-
lar risk (56). By contrast, the cytokine gene polymorphism is associated with vascular injury (57), whereas CRP is most likely only a marker of an inflammatory process. This observation, however, does not exclude the direct effect of other acute phase proteins or changes in lipoprotein concentration, structure, or function in causing vascular injury.

Inflammation Mortality and Malnutrition in the ESRD Patient

The death rate among patients having ESRD is greater than that of many metastatic malignancies (58). The primary causes of death are cardiovascular disease and infection. Inflammation as estimated by measurements of cytokines (59,60) or positive acute phase proteins (61) strongly predicts outcome in dialysis patients. Many of these measurements, specifically cytokines and several of the acute phase proteins such as long pentraxin 3, are research tools and not available clinically to raise suspicion of the presence of inflammation in either a specific dialysis patient or to estimate whether a group of individuals may be challenged by an inflammatory source; however, hypoalbuminemia frequently occurs in response to and indicates the presence of inflammation (45,62).

The laboratory tests or clinical indications that suggest the presence of inflammation that the treating physician encounters without ordering additional laboratory measurements are (1) hypoalbuminemia; (2) decreased transferrin levels; (3) increased neutrophil count; and (4) increasing erythropoietin resistance. Decreasing level of predialysis creatinine (63) and increasing frailty are also observed as an outcome of inflammation, as is decreasing total serum cholesterol concentration. Clearly, measurement of CRP will confirm the presence of inflammation; however, this additional test is not a component of standard monitoring of dialysis patients, requires justification, and incurs cost.

Inflammatory Biomarkers

Hypoalbuminemia

One of the most powerful predictors of mortality in cross-sectional studies is low serum albumin (44). Although protein calorie malnutrition alone may be associated with hypoalbuminemia, in otherwise healthy individuals, serum albumin concentration does not decline until starvation is preterminal (64). Albumin concentration decreases in dialysis patients primarily as a consequence of increased inflammation with a subsequent decline in the rate of albumin synthesis (62) coupled with a failure to downregulate albumin catabolism as occurs during protein restriction. Inadequate dietary nitrogen intake clearly correlates with albumin levels, but nutritional supplements have not been notably effective in correcting hypoalbuminemia, and it is likely that the relationship between nitrogen intake and hypoalbuminemia in dialysis patients is a consequence of an inflammation-mediated inability of these patients to reduce albumin fractional catabolic rate when nitrogen intake is limited that makes these patients sensitive to nitrogen restriction (65). Dialytic amino acid loss may also be a contributing factor.

Both IL-6 (47) and CRP (45,46) have a greater effect than albumin in predicting mortality, and when combined in multiple regression models, inflammatory markers will frequently displace albumin as a predictor of outcome (45), suggesting that it is the inflammatory cause of hypoalbuminemia rather than other causes that dominate the link between hypoalbuminemia and poor outcome in dialysis patients.

Albumin concentration in dialysis patients is negatively correlated with levels of positive acute phase proteins. These are proteins whose synthesis and serum concentration increase as a consequence of increased synthesis during inflammation [CRP, SAA (66,36), fibrinogen, ceruloplasmin, and α1 acid glycoprotein; Figure 1], an increase that is signaled by increase in the cytokines (IL-6, TNFα) (67,68). These markers of inflammation are statistically powerful determinants of current and of future albumin level in ESRD patients (69).

Neutrophil count is strongly associated with CRP (70), and total neutrophil count and neutrophil/lymphocyte ratio are associated with outcome and are evidence of the inflammatory response (71) (Figure 1). Owen and Lowrie (70) concluded that albumin and total lymphocyte count had greater power that CRP; however, their study was of short duration (6 mo), and in our experience, those patients with very low albumin levels (<3.0 g/dl) exhibited a high rate of early mortality (50% in 6 mo) (45), obscuring later events that are predicted by CRP.

Erythropoietin Resistance and Inflammation

Anemia occurs in patients with kidney failure in large part because of the decrease in erythropoietin that results from loss of its major biosynthetic organ. The requirement for the use of ESAs varies widely. Patients having consistently low hemoglobin levels (<11 g/dl) experience the highest percentage of hospitalization admissions, highest percentage of admissions for infection, longest hospital stays, and the highest number of comorbid conditions (72).

Inflammation induces ESA resistance through a number of processes, but the predominant one is that of augmentation of inflammatory cytokines and acute phase proteins to injurious clinical outcomes, including erythropoietin resistance, loss of muscle mass with resulting frailty, and cardiovascular injury.

Figure 1. The relationship between causes of inflammation in dialysis patients and the pathway through inflammatory cytokines and acute phase proteins to injurious clinical outcomes, including erythropoietin resistance, loss of muscle mass with resulting frailty, and cardiovascular injury.
Dyslipidemia

Although cardiovascular disease is the leading cause of death among dialysis patients, neither total cholesterol nor LDL-cholesterol is positively associated with mortality. Indeed, the opposite is true (44). Patients having the lowest level of LDL and total cholesterol are at the greatest risk. At least part of the reason for this is the effect that inflammation has on altering lipoprotein levels and distributions (81,82), so that the effect of high levels of inflammation provide more of an effect on increasing the risk of cardiovascular death than does the effect of a high LDL-cholesterol level in a presumably uninfamed dialysis patient (81) (Figure 1). LDL is oxidized by neutrophil myeloperoxidase, and HDL, which would normally serve as a reducing agent, becomes dysfunctional by the replacement of apolipoprotein CIII by serum amyloid A, an acute phase protein (54), and reducing agent, becomes dysfunctional by the replacement of eloperoxidase, and HDL, which would normally serve as a

ESA resistance is associated with infectious hospitalizations (79) and use of tunneled dialysis catheters (80). Attempts to reduce exposure to bacterial products by improving the quality of water systems have been found to increase ESA sensitivity in groups of dialysis patients, either by providing ultrapure dialysate (31) or even by upgrading the plumbing in an existing dialysis unit (33).

Frailty

Other factors that normally are used to diagnose malnutrition are associated with inflammation. Stenveinkel et al. (86) established that patients with pre-ESRD who were judged to be malnourished by measurement of subjective global assessment also had markers consistent with the presence of inflammation (87). Both CRP and fibrinogen were significantly greater in groups of patients with subjective global assessment ≥ 2. The prevalence of vascular disease judged by prevalence of carotid plaques and elevated calculated intima-media area was also increased in this cohort. Inflammation causes a decrease in muscle mass as a consequence both of increased catabolism of muscle protein and impedance of muscle protein synthesis, including both skeletal and cardiac muscle (88,89) (Figure 1). Because muscle is the primary organ that synthesizes creatinine, low serum creatinine levels are associated with decreased muscle mass, and inflammation is associated with serum creatinine levels in dialysis patients both by cross-sectional analysis (63) and by observing the relationship between creatinine and evidence of inflammation, such as CRP, longitudinally (63), compatible with a loss of muscle mass occurring during or after an episode of inflammation. Frailty is identified by a composite construct that incorporated poor self-reported physical functioning, exhaustion/fatigue, low physical activity, and undernutrition and is associated with CRP and IL-6 levels in subjects without renal failure (90). The frailty phenotype also strongly predicts mortality among HD patients (91).

What Potential Treatment Options Are Present?

Inflammation should be suspected in patients with hypoalbuminemia and ESA resistance, even when characterized by low serum iron, because inflammation blocks iron uptake by the gut and redistribution through the reticuloendothelial system (92). The onset of malnutrition identified by decreased physical function and performance, loss of muscle mass, and decline in serum creatinine should also increase suspicion of the new onset of an inflammatory process. Although CRP is likely not the cause of vascular injury, it is highly regulated by cytokines that are, and it is the most readily available clinical test to directly assess the presence in inflammation. If ESA utilization in a dialysis unit is tending to increase, analysis of a unit-based exposure, such as water quality, should be considered. Infections or biofilm contamination are clearly associated with tunneled dialysis catheters.

Removing tunneled dialysis catheters and replacing them with fistulas should be a priority for every patient in whom this is feasible. If this is not possible, use of AV grafts should be considered. All efforts should be made to get the plastic out! Specific sources for infection including periodontal disease and clotted prior vascular access sites (24) should be explored.

Certain outcomes associated with inflammation, such as ESA resistance, have shown improvement using a variety of interventions including use of biocompatible membranes (93), ultrapure dialysate (31), improved water system (33), transplant nephrectomy (94), ascorbic acid therapy (95), and vitamin E–coated dialyzers (96). However, at this time, there is no firm
basis to recommend nonspecific anti-inflammatory therapy or anti-oxidant dietary or vitamin supplementation as a way of combating inflammation (97). Therapy should be targeted at identification of potential sources of inflammation and removal of those sources.

Visceral adiposity is a source of inflammatory cytokines (34,35) and is a risk factor for loss of renal function (98,99). Some small studies have reported a renoprotective effect of weight reduction after gastric bypass surgery (100,101). Gastric bypass surgery has been used in nonrandomized studies in several patients with CKD (102), leading to a sustained decline in BMI and either a decline in proteinuria or stabilization of renal function. However, intestinal bypass surgery is associated with increased urinary oxalate excretion and increased risk of renal stones and even acute renal failure (103,104). Perioperative acute renal failure has also been reported (105), as has rapid progressive decline in renal function, resulting in end-stage renal failure. Thus, the effect of these procedures on protection of renal function is at best less than certain.

Disclosures

None.

References


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