Author's response to reviews

Title: Neutrophil Gelatinase-Associated Lipocalin (NGAL) predicts Renal Injury in Acute Decompensated Cardiac Failure: A prospective observational study

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Author's response to reviews: see over
Please see detailed responses to reviewer comments below.

Reviewer's report

Title: Neutrophil Gelatinase-Associated Lipocalin (NGAL) predicts Renal Injury in Acute Decompensated Cardiac Failure: A prospective observational cohort study

Version: 1 Date: 7 November 2011

Reviewer: David R McIlroy

Reviewer's report:

Major compulsory revisions:

1. Background section. How common is AKI in patients admitted with ADCF? More detail is required on presumed etiology of AKI in ADCF and why early diagnosis may be important. Would such knowledge potentially change initial management from diuretic-based to inotropic-based? The authors should make a stronger case for why we need recognition of evolving AKI at admission.

The background section has been revised in accordance with these suggestions

2. Methods: Please make explicit that NGAL was measured in blood only and not measured in urine.

A sentence has been added to this effect

Were NGAL results corrected for urinary creatinine? If not, please justify. These patients may have had significant variation in urine flow at time of presentation to the E.D.

We did not examine urine NGAL, only plasma. Correction for urinary creatinine is relevant for urine NGAL.

In terms of the sample size determination, what was an "elevated NGAL" and how was this determined.

We used >149ng/ml, the manufacturer quoted 95th percentile as “positive” to define elevated NGAL for the purposes of the sample size calculation. This is now made clearer in the methods.

Results: Why didn't 12 patients have subsequent data? This should be explained. Did they die shortly after admission? Did they get lost?

These patients were enrolled and then transferred to another hospital where they could not be followed up, or did not have any further testing for renal function while in hospital. One patient died before they could have follow up blood testing. It was not possible therefore to classify these patients for the primary outcome. The results section has been amended accordingly to clarify this.

On follow up, how may patients were confirmed to have the correct diagnosis if
The hospital discharge summary was examined. In four patients of the 90 examined for the primary outcome, the discharge diagnosis was subsequently thought not to be ADCF. All of these patients had NGAL <89ng/ml and none developed AKI. Recalculation of the sensitivities and specificities of NGAL for AKI when these patients were excluded made negligible difference. This information has been added to the results section.

More information is required for the methodology of the multivariable analysis. A general rule-of-thumb is that there should be at least 8-10 outcomes for each explanatory variable in the model? With 22 events (AKI) in this study there is limited scope for a multivariable regression analysis. This needs to be addressed.

This is a valid point and this does limit the use of a multivariate regression model, given the sample size. On univariate analysis, only age and eGFR at presentation are significantly associated with risk of AKI. Other potential confounders (IV contrast, diuretic dose) were not found to be significant (probably due to small numbers). We therefore have removed the multivariate regression analysis and instead fitted logistic models with only NGAL and eGFR (which includes age in its calculation). Models were fitted for NGAL >89ng/ml and >149ng/ml and eGFR was fitted as both a continuous and categorical variable, with eGFR<60ml/kg/1.73m² defining renal impairment as a binary variable. The adjusted OR for NGAL at the lower cut point was 3.31 (1.08-10.14) and 3.73 (1.26-11.01) when the model is fitted with eGFR as a continuous and categorical variable respectively. At the higher cut point, OR for AKI was 4.25 (1.30-13.59) and 4.78 (1.49-15.30) respectively with eGFR fitted as a continuous and categorical variable. However, the model fit was poor for the lower NGAL cut point with eGFR as a continuous variable (Hosmer & Lemeshow p value 0.034). Therefore, the OR for NGAL after adjustment for eGFR<60 as a categorical variable is quoted in the paper for both cut points. This has been added to the text of the results section and the table describing the multivariate regression analysis removed.

Discussion: Please discuss why AKI might be associated with increased mortality in this study but not increase length of stay.

Most likely this was a chance finding, given the small numbers. It is also credible that patients who died had shorter length of stay in hospital prior to death. However, the mean length of stay was not significantly different in those who died from those who did not (8.14 days vs 7.01 days, p=0.952).

How did your results vary if the analysis was repeated in a subset with physician-confirmed ADCF?

See above. Four patients who were analysed for the primary outcome were found subsequently not to have evidence of ADCF. These all had BNP <400pg/ml, low NGAL levels (below cut points defined above) and none developed AKI. When excluded, the sensitivity for AKI did not therefore change, and there was only minimal change in specificity. For the 89ng/ml cut point, sensitivity was 68% and specificity was 69% after exclusion of these patients.

What was the incremental benefit of admission NGAL (expensive) over and above admission eGFR (very cheap) for identifying risk of AKI.
When NGAL is adjusted for admission eGFR it is an independent predictor of AKI, and thus does add incremental value in identifying a high risk population. However the key is whether this is useful in the individual patient, and whether it changes outcome, these questions requires further investigation, as we state in our conclusion.

Is there any evidence that NGAL discriminated patients with so-called pre-renal azotemia from patients with true acute tubular injury. This is an interesting suggestion, however this was not the primary purpose of the study. Accurately differentiating ATN from prerenal azotemia requires investigations such as fractional sodium excretion, urine microscopy, renal ultrasonography and response to fluid challenge etc, which were beyond the scope of the study.

The conclusion does not seem to fit with the data. I would suggest that the data indicate that NGAL is a useful and expensive measure of admission GFR, but that no incremental benefit over and above this simple measure has been demonstrated. Please address this.

Of 22 who developed AKI, 19 had admission eGFR <60. Of these 14 (74%) had an elevated NGAL >89ng/ml, compared to one of 3 patients with normal admission eGFR who developed AKI. Low eGFR at admission, found in the majority of patients in the study, is associated with high risk for developing AKI. NGAL appears to discriminate AKI from chronic renal impairment in this group of patients, which is borne out by the adjusted odds ratio for elevated NGAL when eGFR is taken into account. We therefore conclude that NGAL does add incremental value over admission eGFR in identifying a high risk group for AKI in hospital (ie worsening renal function after admission), but the sensitivity and specificity are only moderate, therefore limiting its utility in the individual case. This is likely due to AKI being multifactorial in aetiology and the timing of the renal insult varies in relation to timing of presentation to care in ADCF, unlike other settings such as anaesthesia. In addition we conclude that the effect of knowledge of NGAL level on outcome is uncertain. Cost-effectiveness clearly depends upon whether doing this test can prevent AKI, and reduce related costs. This is unknown. We have rephrased the conclusion to emphasise this uncertainty.

Reviewer’s report
Title: Neutrophil Gelatinase-Associated Lipocalin (NGAL) predicts Renal Injury in Acute Decompensated Cardiac Failure: A prospective observational cohort study

Version: 1 Date: 10 November 2011

Reviewer: Jean-Michel Constantin

Reviewer’s report:
In this prospective observational study, Macdonald have investigated if NGAL measured at presentation predicts in-hospital acute kidney injury in Acute Decompensated Cardiac Failure. The question raised is of interest but we have serious concerns with this manuscript.

First of all, predictive positive and negative values presented are inferior to data
shown in peri-operative cardiac patients or ICU patients. This is not a concern for us, and we think it must be discussed. But before discussing, you probably have to define better what you think AKI is. You used RIFLE, but without urine output, please justify.

We recorded urine output and weight daily for patients in addition to serum creatinine. The RIFLE-R (Risk) category requires either a fall in urine output to <0.5ml/kg/hr for at least 6 hours, rise in creatinine >50% or fall in eGFR >25% from baseline. Previous investigators have used a definition of worsening renal function (WRF) typically defined by an absolute rise in creatinine. Our view is that the RIFLE criteria, a validated tool for identifying renal injury, were more physiologically appropriate as this takes account of patient age and weight.

When did your patients develop AKI, at day 1, day 2 day …5? If patients developed AKI due to an injection of intravenous contrast at day 4, it is comprehensible that NGAL at admission was normal.

Patients were followed for up to 7 days. Of the 22 who developed AKI, 13 (60%) met criteria within 48 hours of admission, the remainder thereafter. We would caution against over interpreting this however. We postulated that AKI is a dynamic condition with multiple causes in this setting, including possibly iatrogenic. As we discuss, some patients presenting with high NGAL levels did not experience worsening renal function in hospital, and in fact had an improvement in their eGFR. These patients likely had AKI, but did not meet our a priori definition. These factors make the precise timing of AKI difficult to ascertain. Knowledge of recent baseline renal function when well could assist with this, as would serial NGAL estimation which would go some way to resolve this. Emergency Physicians do not always have this information when treating the patient in front of them. Our pragmatic study design reflects this reality and our overall aim was to determine the utility of NGAL for in hospital AKI regardless of timing or postulated cause.

NGAL is a biomarker of AKI, validated in different situations, but NGAL do not increase in case of pre-renal azotemia. What is the amount of pre-renal azotemia in your patients, how did you splitted AKI and pre-renal azotemia?

Please see response to reviewer 1 above

One other possibility to explain your results is the amount of chronic kidney disease in this old population. Please show us some data.

We agree. 60% of patients had admission eGFR <60ml/min/1.73m2 (table 1). This is consistent with other studies of heart failure (see reference 4). The problem is whether this represents chronic or acute renal impairment at time of presentation to hospital, and hence the purpose of the study to determine whether NGAL can assist to differentiate. We excluded patients with end stage renal failure on renal replacement therapy from enrolment. 45% of patients had a history of diabetes and 68% hypertension (Table 1) so chronic renal impairment is likely to be prevalent also.

Method and result section are difficult to understand, please rephrase.

It is not clear which particular aspects are of concern. We consider these sections to be a clear and concise account of the study.
NGAL is an ubiquitous peptide, please change the sentence “Neutrophil gelatinase-associate lipocalin (NGAL), produced by renal tubular cells… » in the abstract, the body of the manuscript …

This has been amended.

You said that NGAL is a biomarker released by renal tubular cells in response to injury, has been shown to be an early marker of acute kidney injury (AKI) in a number of settings,[5-8 of yours references]. Please cite only original papers, try to cite papers with plasma NGAL and adults patients, it will be more accurate according to your population than urinary and paediatric NGAL.

We have removed irrelevant references and now cite only original papers examining blood NGAL in adults.

Level of interest: An article of importance in its field
Quality of written English: Acceptable
Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.
Declaration of competing interests:
I received grants from Alare to be the coordinator of a study

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Perth, Western Australia, 14 December 2011