Neutrophil gelatinase-associated lipocalin as a risk marker in cardiovascular disease

Zenthuja Sivalingam, Sanne Bøjet Larsen, Erik Lerkevang Grove, Anne-Mette Hvas, Steen Dalby Kristensen and Nils Erik Magnusson*

https://doi.org/10.1515/cclm-2017-0120
Received February 10, 2017; accepted May 7, 2017; previously published online June 23, 2017

Abstract: Neutrophil gelatinase-associated lipocalin (NGAL) is a promising diagnostic biomarker of early acute kidney injury. Increasing evidence suggests that NGAL may also be involved in inflammatory processes in cardiovascular disease. NGAL modulates the enzymatic activity of matrix metalloproteinase-9 (MMP-9), which is an important mediator of plaque instability in atherosclerosis. The complex formation between NGAL and MMP-9 therefore suggests that NGAL might play a role in progression of atherothrombotic disease. This review summarises current data on NGAL in atherosclerosis, acute myocardial infarction, and heart failure.

Keywords: atherosclerosis; biomarkers; cardiovascular disease; inflammation; neutrophil gelatinase-associated lipocalin (NGAL).

Introduction

Coronary artery disease (CAD) is caused by atherosclerosis and is the main cause of mortality worldwide [1]. Atherosclerosis is regarded a chronic vascular inflammatory disease with a complex multifactorial pathophysiology. Inflammation within the atherosclerotic plaque has been suggested to promote plaque instability and disruption [2].

Previously, elevated levels of high-sensitive C-reactive protein (hs-CRP) have been associated with a 45% increased risk of cardiovascular events in patients without established CAD [3]. In patients with CAD, increased hs-CRP is considered a risk marker of adverse events [4]. Thus, inflammatory biomarkers may have the potential to improve risk assessment in CAD patients.

It has been hypothesised that neutrophil gelatinase-associated lipocalin (NGAL) may be a marker of atherosclerosis, which is supported by recent data demonstrating excessive expression of NGAL in atherosclerotic plaques in areas with high proteolytic activity [5]. The role of NGAL in CAD patients has been suggested to involve matrix metalloproteinase-9 (MMP-9) [5]. Other studies report that NGAL may be a risk marker in heart failure (HF) patients [6] and in patients undergoing cardiac surgery [7].

In this review, analytical approaches and the role of NGAL in cardiovascular disease (CVD) will be summarised and discussed focusing on the potential role of NGAL as a risk marker of atherosclerosis.

Materials and methods

In January 2017, we conducted a systematic literature search on PubMed. The search was carried out both on basis of medical subject headings (MESH) tree and as a text search, and it was limited to English language and year of publication, ranging from 2000 to 2017, using the MESH terms “Neutrophil gelatinase-associated protein and coronary artery disease”, “NGAL and cardiovascular disease”, and “NGAL assay”. In addition, reference lists were studied, and manual searches performed. The primary focus of this review is to outline and summarise the characteristics of NGAL in CVD. Consequently, the review is limited to clinical trials and experimental studies in cardiac settings. So far, many studies have demonstrated NGAL as a novel biomarker for early diagnosis
of acute kidney injury (AKI). Recent studies suggest that NGAL is a promising biomarker of CVD and is hypothesized to reflect the degree of atherosclerotic inflammation in patients with CAD.

**Main characteristics of NGAL**

NGAL is a 25 kilodalton (kDa) human protein belonging to the lipocalin superfamily [8]. The human neutrophils are the main source of plasma NGAL, which are constitutively expressed and synthesized at early-myelocyte stages of granulopoiesis in the bone marrow [9]. Consequently, the mature peripheral neutrophils only store NGAL protein in granules.

It has been demonstrated that NGAL functions as an inflammatory modulator of the innate immune system [10]. This is supported by the fact that NGAL is capable of binding siderophores, reducing the amount of siderophore-bound iron available to bacteria inhibiting bacterial growth by depleting their intracellular iron stores [11].

The complex between NGAL and MMP-9 inhibits degradation of MMP-9 thereby extending the proteolytic activity of MMP-9 (Figure 1) [13]. By enhancing the activity of MMP-9, the degradation of the basement membranes and extracellular matrix increases, a process recognised in the progression of unstable atherosclerotic plaques in arteries [5]. In atherosclerotic plaques, the collagen degradation by MMP-9 is high, which causes an unstable fibrous cap and makes the atherosclerotic plaque more susceptible to erosion and rupture [5].

**Assessment of NGAL levels in blood and urine**

Differences in NGAL cut-off levels for prediction of outcome in diseases and reference values in the healthy population have been observed in numerous reports [14]. Several factors contribute to the variation such as different clinical settings, biological variation, use of different assays, antibody configurations, anticoagulants, and storage conditions [15]. Different methods including commercial and in-house sandwich immunoassays and immunoblotting protocols have been used to determine NGAL in plasma, serum, and urine [16–18].

Pedersen et al. tested the use of enzyme-linked immunosorbent assay (ELISA) kit, using the manual procedure, (BioPorto Diagnostics) for NGAL in plasma and urine. In
healthy adults, the median intra and inter-assay variation was <5% and <10% for plasma and urine, respectively [19].Similar results have been reported in other studies [18,20].In the study by Pedersen et al., the highest inter-assay variation in urine was ≤18.6% and likely explained by interference of sedimentation factors, emphasising the importance of centrifuging urine samples before analysis. Moreover, in plasma it was demonstrated that haemolysis caused NGAL levels to increase, which may be explained by release of NGAL from ruptured leucocytes, a process also observed in serum samples following multiple freeze/thaw cycles. In plasma and urine NGAL samples, levels were not significantly affected by repetitive freeze/thaw cycles [19].

To accommodate standardised assays for inclusion of NGAL in the standard panel to screen kidney diseases, the analytical performance of automated biochemistry analysers have been evaluated (Table 1). Cavalier et al. [32] performed an analytical comparison between a rapid bedside fluorescence-based immunoassay NGAL method (Triage Meter, Biosite Inc.) and the automated Architect NGAL laboratory assay with ethylenediaminetetraacetic acid (EDTA) plasma and urine samples. Both assays correlated well with validated ELISA kits from BioPorto. The Architect assay was shown to provide precise results in the range 22.5-1315 μg/L with maximum error of ±20% and probability of 95%. Using the same conditions, the corresponding range for the Triage method was 619-722 μg/L, indicating high analytical variation of the Triage method [32].

Hansen et al. [20] evaluated the turbidimetric NGAL Test™ from BioPorto with an automated method, Cobas 6000 c501 (Roche Diagnostics, Rotkreuz, Switzerland). The analytical performance was comparable to the automated Hitachi 917 in 40 matched samples of urine, Li-Hep plasma, and EDTA plasma. It was noted that NGAL levels were higher in Li-Hep plasma than in EDTA plasma, thus EDTA was recommended as anticoagulant.

Delanaye et al. [29] studied the biological variation of NGAL by measuring the absolute value of urinary NGAL and NGAL/creatinine ratio using an automated immunoassay from Abbott Laboratories (Abbott Park, IL, USA) on the Architect platform. As the coefficient of variation (CV) for urinary NGAL was higher than the CV of NGAL/creatinine ratio, the authors recommended the use of NGAL/creatinine ratio to reduce intra-individual variation. Urinary NGAL variation was measured in healthy subjects only, thus the biological variation in patients with AKI or CVD remains unknown.

While several reference ranges for urine NGAL have been published, reference ranges for plasma NGAL have not yet been fully explored. Published reference ranges are summarised in Table 1.

NGAL and cardiovascular disease

NGAL in atherosclerotic plaques

In atherosclerotic plaques, thrombus formation is triggered by rupture of the plaque cap or endothelial erosion. Inside the plaque, inflammatory activity facilitates plaque instability and disruption [2]. The atheroma possesses inflammatory mediators, which inhibit smooth muscle growth and collagen production, and enhance the activity of MMP’s [12]. This also makes the atherosclerotic plaques unstable and vulnerable, potentially leading to plaque rupture.

Hemdahl et al. [5] analysed the association between NGAL expression in atherosclerotic plaques and myocardial infarction (MI) in mice exposed to hypoxic stress. Mice developing MI had higher concentrations of NGAL and MMP-9 than mice with stable atherosclerotic plaques. In atherosclerotic plaques retrieved from human carotid endarterectomy specimens, expression of NGAL and MMP-9 were detected in macrophages [5]. Yndestad et al. found that NGAL was over-expressed in the myocardium following MI in a rat model, and murine data suggests that mice deficient in the NGAL mouse orthologue were protected from development of aortic aneurysms [6,33].

In patients undergoing surgery for infrarenal abdominal aortic aneurysms, NGAL/MMP-9 complexes were identified in the thrombus and in the interface between the thrombus and the underlying wall [34]. Furthermore, NGAL has also been associated with intraplaque hemorrhages of human carotid endarterectomy samples [35].

Although causality is not explicitly proven, these findings might suggest a pro-atherogenic action of NGAL via enzymatically active NGAL/MMP-9 complexes. However, further studies are needed to explore whether NGAL is an important and specific marker of atherosclerosis.

NGAL and acute coronary syndrome

The association between NGAL levels and risk scores (clinical and angiographical) has been studied in patients diagnosed with non-ST-segment elevation MI (NSTEMI) and compared with healthy individuals [36]. High levels of NGAL, hs-CRP, and leukocytes were observed in the patient group. Furthermore, NGAL correlated positively
Table 1: Neutrophil gelatinase associated-lipocalin studies on reference ranges included in this review.

<table>
<thead>
<tr>
<th>Study (reference)</th>
<th>Study population, n (age range, years)</th>
<th>Assay, (specimen)</th>
<th>Limit of quantification</th>
<th>Reference range (uNGAL normalised with urinary creatinine)</th>
<th>Reference range (absolute values)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Makris et al. [21]</td>
<td>Healthy adults, 137 males and 63 females (18–65)</td>
<td>Particle-enhanced turbidimetric immunoassay, the NGAL Test™ (Bioporto, Gentofte, Denmark) on the Architect c8000 platform (plasma and urine)</td>
<td>Plasma: 8.4 ng/mL Urine: 9.0 ng/mL</td>
<td>N.R.</td>
<td>Plasma NGAL (significantly higher pNGAL in men than women, p = 0.02) Lower-upper limits: Male: 38.7–157.6 ng/mL Female: 24.4–142.5 ng/mL Urine NGAL (no significant difference in uNGAL between men and women, p = 0.12) Lower-upper limits: Both genders: &lt;9–54.5 ng/mL</td>
</tr>
<tr>
<td>Cullen et al. [22]</td>
<td>Healthy adults, 100 men and 74 women (19–88)</td>
<td>Abbott ARCHITECT i1000sr system (Abbott Park, IL, USA) (urine)</td>
<td>Urine: 10 ng/mL</td>
<td>N.R.</td>
<td>Urine NGAL (significantly higher uNGAL in women than men, p = 0.007) 95th centile reference intervals: Male: 91 μg/L Female: 129 μg/L</td>
</tr>
<tr>
<td>Zwiers et al. [23]</td>
<td>Healthy infants, 71 boys and 35 girls (1 day to 1 year)</td>
<td>Immunoassay, ARCHITECT analyser (urine)</td>
<td>Urine: 3.0 ng/mL</td>
<td>95th centile reference intervals: Boys: 49.7 ng/mg Girls: 96.1 ng/mg</td>
<td>Urine NGAL (significantly higher uNGAL in girls than boys, p &lt; 0.001) 95th centile reference intervals: Boys: 72.3 ng/mL Girls: 136.9 ng/mL</td>
</tr>
<tr>
<td>Cangemi et al. [24]</td>
<td>Healthy newborns, 16 boys and nine girls (1–4 days) Healthy children, 150 boys and 147 girls (0.63–248 months)</td>
<td>ARCHITECT NGAL assay (Abbott, Diagnostic Italia, Rome, Italy) (urine)</td>
<td>Urine: 0.95 ng/mL</td>
<td>Mean: 23.4 ng/mg, SD: 58.8 97.5th centile reference intervals: All subjects: 135.8 ng/mg</td>
<td>Significantly higher uNGAL levels in the neonatal population than older children, p &lt; 0.0001. No significant difference in uNGAL between genders. Mean: 12.7 ng/mL, SD: 21.3 97.5th centile reference intervals: All subjects: 88.6 ng/mL</td>
</tr>
<tr>
<td>Mcwilliam et al. [25]</td>
<td>Healthy children, 64 boys and 56 girls from UK and 108 boys and 63 girls from US (UK: birth to 16 years) (USA: 7–16 years)</td>
<td>Electrochemiluminescent assays (Meso Scale Discovery, MD, USA) (urine)</td>
<td>Urine: 0.041 ng/mL</td>
<td>97.5th centile reference intervals for patients aged 1–4 to 13–16 years: UK boys: 229.9–111.3 ng/mg UK girls: 706.7–394.1 ng/mg 97.5th centile reference intervals for patients aged 7–8 to 13–16 years: USA boys: 8.3–14.0 ng/mg USA girls: 58.3–191.2 ng/mg</td>
<td>N.R.</td>
</tr>
<tr>
<td>Study (reference)</td>
<td>Study population, n (age range, years)</td>
<td>Assay, (specimen)</td>
<td>Limit of quantification</td>
<td>Reference range (uNGAL normalised with urinary creatinine)</td>
<td>Reference range (absolute values)</td>
</tr>
<tr>
<td>-------------------</td>
<td>----------------------------------------</td>
<td>-------------------</td>
<td>-------------------------</td>
<td>----------------------------------------------------------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td>Bennett et al. [26]</td>
<td>Healthy children, 184 boys and 184 girls (3–18)</td>
<td>NGAL ELISA kit 036, (Bioporto, Grusbakken, Denmark) (urine)</td>
<td>N.R.</td>
<td>N.R.</td>
<td>Urine NGAL (significantly higher uNGAL in girls than boys, p &lt; 0.001) 95th centile reference intervals: Boys: 28.3 ng/mL Girls: 73.1 ng/mL</td>
</tr>
<tr>
<td>Pennemans et al. [27]</td>
<td>Healthy individuals, 199 women and 139 men (0–95)</td>
<td>NGAL kit (RD Systems Europe, Abingdon, UK) (urine)</td>
<td>N.R.</td>
<td>95th centile reference intervals for patients aged 0–10 to +80 years: Male: 167.3–307.8 μg/g Female: 325.4–598.3 μg/g</td>
<td></td>
</tr>
<tr>
<td>Rybi-Szumińska et al. [28]</td>
<td>Healthy children and adolescents, 88 men and 84 women (0.2–17.9)</td>
<td>ELISA kit (R&amp;D Systems, Minneapolis, MN, USA) (urine)</td>
<td>0.012</td>
<td>Male: median 1.7 ng/mg (range: 0.01–33.9) Female: median 2.5 ng/mg (range: 0.05–48.79) 97.5th centile reference intervals for patients aged 0.2–5.9 to 14–17.9 years: All subjects: 33.91–15.69 ng/mg</td>
<td>97.5th centile reference intervals for patients aged 0–10 to +80 years: Male: 52.9–211.2 μg/L Female: 141.8–182.6 μg/L</td>
</tr>
<tr>
<td>Delanaye et al. [29]</td>
<td>Healthy adults, 20 (Mean: 33.3)</td>
<td>Abbott ARCHITECT i1000sr system (Abbott Park, IL, USA) (urine)</td>
<td>N.R.</td>
<td>N.R.</td>
<td>First morning sample: median 29.2 ng/mL (IQR 15.4–52.8) Second morning sample: median 38.5 ng/mL (IQR 20.6–70.9)</td>
</tr>
<tr>
<td>Huynh et al. [30]</td>
<td>Premature newborns, 30 boys and 20 girls (4–30 days)</td>
<td>Immunoblotting, polyacrylamid gels (Bio-Rad) (urine)</td>
<td>N.R.</td>
<td>N.R.</td>
<td>95th centile reference intervals: Male: 20 ng/mL Female: 100 ng/mL</td>
</tr>
<tr>
<td>Stejskal et al. [31]</td>
<td>Healthy adults, 53 men and 83 women (mean: 62.8)</td>
<td>Sandwich ELISA (serum)</td>
<td>Serum: 0.02 μg/L</td>
<td>N.R.</td>
<td>Serum NGAL (no significant difference in serum NGAL between females and males, p = 0.56)</td>
</tr>
</tbody>
</table>

NGAL, neutrophil gelatinase-associated lipocalin; ELISA, enzyme-linked immunosorbent assay; N.R., not reported; uNGAL: urinary NGAL; IQR: interquartile range.
with the global registry of acute coronary events (GRACE) risk score (which estimates the 6-month mortality risk in patients with acute coronary syndrome [ACS]). The severity of CAD was assessed by the Gensini score (evaluates collateral circulation of the coronary arteries) and the synergy between percutaneous coronary intervention with taxus and cardiac surgery (SYNTAX) (estimates the complexity of CAD) score showing increased NGAL levels in patients with intermediate and high SYNTAX scores compared to patients with low SYNTAX score [36].

Sahinarslan et al. reported that patients with acute MI had higher levels of plasma NGAL and leukocytes than stable CAD patients [37]. However, there was no correlation between plasma NGAL and the Gensini score. These findings were supported by Choi et al., who reported that NGAL levels were higher in patients with acute MI and unstable angina pectoris than stable CAD patients. However, the findings were not statistically significant, possibly due to a low number of patients diagnosed with ACS (n = 11). Also, blood samples were collected at least 3 months after the acute event [38].

Akcay et al. [39] reported a poor prognosis in patients with high NGAL levels and ST-segment elevation MI (STEMI) treated with primary percutaneous coronary intervention (PCI) compared to patients admitted for elective PCI. Similarly, high NGAL levels independently predicted all-cause mortality and major adverse cardiac events (MACE) in STEMI patients treated with PCI [40]. Furthermore, the study showed that high levels of CRP and NGAL were related to poor outcomes, while low levels of NGAL and CRP were associated with decreased risk of MACE. Indeed, the risk still remained high when NGAL was elevated even though CRP was low [40]. This is consistent with findings by Zykov et al., who also reported that high NGAL level, is a strong predictor of all-cause mortality suggesting a high prognostic value of NGAL in patients with STEMI [41].

The majority of these studies (Table 2) suggest that NGAL has the potential to improve risk stratification in patients with ACS.

NGAL in stable coronary artery disease

Only few studies have measured NGAL in stable CAD patients (Table 2), and so far, no causal relation between NGAL and stable CAD has been established. Paulsson et al. [42] compared levels of neutrophils and MMP-9/NGAL complexes in stable CAD patients with age- and sex matched healthy individuals. Neutrophil levels did not differ between patients and healthy individuals, neither in peripheral blood nor at the site of intense inflammation.

However, the concentration of interleukin-8 (IL-8), which stimulates the release of NGAL from neutrophil granules, was higher in patients than healthy individuals. This may contribute to the observed higher concentration of the MMP-9/NGAL complex in patients than healthy individuals and suggest a priming stage of circulating neutrophils in stable CAD patients [42].

Zografos et al. [43] reported that serum NGAL levels in stable CAD patients were significantly increased compared with patients with normal coronary arteries. Another study showed that patients with triple vessel disease had higher serum levels of NGAL than patients with single vessel disease [46]. Moreover, a positive association between NGAL and the SYNTAX score was reported. Patients with high levels of NGAL (>100 ng/mL) and brain natriuretic peptide (BNP) (>25 pg/mL) had a higher SYNTAX score than patients with low levels of NGAL (<100 ng/mL) and BNP (<25 pg/mL) [46].

Recently, Woitas et al. analysed the predictive role of plasma NGAL in CAD patients, including unstable and stable CAD patients and reported that plasma NGAL levels were independently related to all-cause mortality as well as CVD mortality after adjusting for conventional cardiovascular risk factors. However, when adjusting for creatinine levels, plasma NGAL did not predict all-cause mortality and CVD mortality [44]. Thus, the studies indicate that NGAL levels are higher in patients with ACS than in stable CAD patients, and that creatinine is a strong predictor of mortality [37, 45].

NGAL levels and acute kidney injury in cardiac surgery

AKI after cardiac surgery is a relatively frequent event, which causes dialysis, prolonged hospitalisation and increased in-hospital mortality [47]. A short summary of the studies on cardiac surgery and AKI are listed in Table 3.

In one study, 21 children underwent cardiac surgery on extracorporeal circulation and developed postoperative AKI. A significant increase in urinary NGAL concentration was reported in these children [16]. Others found that plasma NGAL concentration tripled and remained significantly elevated in 120 children who developed AKI after cardiac surgery [48].

Similar findings have been reported in studies on adults. In patients who developed AKI after cardiac surgery, urinary NGAL peaked within 2–4 h postoperatively, whereas the concentration of serum NGAL did not increase significantly [47]. Tuladhar et al. [17] also studied the development of AKI after cardiac surgery. The plasma
Table 2: Summary of neutrophil gelatinase-associated lipocalin studies including patients with coronary artery disease.

<table>
<thead>
<tr>
<th>Study (reference)</th>
<th>Study population, n (Mean age, years)</th>
<th>Specimen and method</th>
<th>Results</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soylu et al. [36]</td>
<td>NSTE-ACS, 47 (57)</td>
<td>Serum ELISA</td>
<td>Higher serum NGAL in patients than controls with normal coronary arteries.</td>
<td>NGAL was positively associated with CAD severity in patients with NSTE ACS. NGAL was strongly associated with GRACE score and moderately associated with SYNTAX score.</td>
</tr>
<tr>
<td>Sahinarslan et al. [37]</td>
<td>Clinical diagnosis of CAD (STEMI, NSTEMI or stable CAD), 128 (AMI = 59 Stable CAD = 59)</td>
<td>Plasma ELISA</td>
<td>Higher NGAL levels in ACS patients than in stable CAD patients. No significant difference in plasma NGAL levels between subgroups of AMI.</td>
<td>Plasma NGAL levels may have potential to detect patients with increased risk of AMI.</td>
</tr>
<tr>
<td>Akcay et al. [39]</td>
<td>STEMI, 106 Low-NGAL &lt; 46 ng/mL= 53 High-NGAL &gt; 46 ng/mL = 53 (Low NGAL = 52 High NGAL = 51)</td>
<td>Serum ELISA</td>
<td>Higher serum NGAL levels in STEMI patients than in controls. In patients with high NGAL levels, risk of MACE and death was increased compared with patients with low NGAL levels. AUC = 0.76</td>
<td>High NGAL levels were associated with poor prognosis in PCI treated STEMI patients.</td>
</tr>
<tr>
<td>Lindberg et al. [40]</td>
<td>STEMI treated with PCI, 584 High NGAL &gt; 170.1 µg/L = 146 Low NGAL &lt; 170 µg/L = 438 (High NGAL = 66 Low NGAL = 62)</td>
<td>Plasma Immunofluorometric assay</td>
<td>Higher plasma NGAL level was significantly associated with risk of all-cause mortality and MACE.</td>
<td>High plasma NGAL was associated with all-cause mortality and MACE in PCI treated STEMI patients.</td>
</tr>
<tr>
<td>Zykov et al. [41]</td>
<td>STEMI = 85</td>
<td>Serum ELISA</td>
<td>Patients with serum NGAL levels &gt;2.6 ng/mL had higher risk of mortality compared to STEMI patient with lower NGAL levels (OR: 4.42 [1.30–15.16]).</td>
<td>High serum NGAL levels in STEMI patients were associated with all-cause mortality during 3-years follow-up.</td>
</tr>
<tr>
<td>Choi et al. [38]</td>
<td>CAD patients divided into three groups; AMI, UAP and SAP, 49 SAP = 38 AMI or UAP = 11 (61)</td>
<td>Serum ELISA</td>
<td>Significantly higher serum NGAL levels in CAD patients than healthy controls.</td>
<td>No significant difference between NGAL levels in patients with SAP and AMI/UAP.</td>
</tr>
<tr>
<td>Paulsson et al. [42]</td>
<td>Angiographically confirmed CAD, 13 (59)</td>
<td>Blister fluid/serum Flow cytometer/ Multi- Q-Prep ImmunoPrep technique</td>
<td>Higher MMP-9/NGAL serum concentrations in patients than controls. Only significantly high MMP-9/NGAL concentrations in blister fluid at the site of intermediate inflammation. No difference at the site of intense inflammation.</td>
<td>Circulating neutrophils in CAD patients may present a priming stage in terms of increased levels of MMP-9/NGAL and IL-8.</td>
</tr>
<tr>
<td>Zografos et al. [43]</td>
<td>First-time angiography for suspected CAD, 31 (65)</td>
<td>Serum ELISA</td>
<td>Higher serum NGAL in patients with angiographically confirmed CAD than controls with normal coronary arteries. Significant correlation between serum NGAL levels and number of diseased vessels.</td>
<td>Serum NGAL moderately correlated with the severity of the CAD.</td>
</tr>
</tbody>
</table>
and urinary AUC of NGAL using receiver operating characteristic curves (ROC) were 0.80 and 0.96, respectively. This is in accordance with a study by Koyner et al. showing that the plasma NGAL concentration is inferior to urinary NGAL concentration in predicting AKI [50]. Taken as a whole these studies suggest a relevant role for urinary NGAL as a biomarker of AKI in cardiac surgery patients. However, a recent study by Friedrich et al. demonstrated that urinary NGAL failed to predict AKI in cardiac patients undergoing cardiopulmonary bypass and showed no correlation between urinary NGAL and postoperative AKI classification levels, even though urinary NGAL levels increased after surgery [49].

Although, both urinary and plasma NGAL levels are elevated in clinical conditions with AKI, studies indicate that urinary NGAL may be superior to plasma NGAL in diagnosing and predicting AKI. However, due to heterogeneity of studies and varying diagnostic criteria of AKI, a large variation of diagnostic accuracy has been reported with differing AUC’s (Table 2). Clerico et al. [15] reported AUC’s ranging from 0.54–0.96 for plasma NGAL and 0.61–0.98 for urine NGAL, indicating a large variation in the discrimination of patients with or without AKI. This may mainly be explained by enrolment of low numbers of patients, varying diagnostic criteria for the clinical endpoint AKI, different NGAL assays, non-standardised preanalytical variables (specimen type, storage and anticoagulants), and heterogenic study populations in relation to age and clinical setting.

**NGAL in patients with heart failure**

Growing evidence suggests that inflammation and matrix degradation in the myocardium play a pathogenic role in HF, which may be related to NGAL [6]. A summary of the studies on HF is provided in Table 4.

Yndestad et al. [6] investigated the role of NGAL in 236 patients with acute post-MI HF and 150 patients with chronic HF. Patients in NYHA class III had significantly higher plasma NGAL levels than patients in NYHA class I or II and controls. Additionally, high baseline plasma NGAL levels were associated with an increased incidence of the composite endpoint (non-fatal MI, stroke, cardiovascular death, and all-cause death) at 27 months (median) follow-up. Finally, a significant association between plasma NGAL and N-terminal prohormone of brain natriuretic peptide (NT-proBNP) was reported. Similar results were found by Bolignano et al. [51] in elderly chronic HF patients.

In a recent study, the predictive value of baseline NGAL was assessed in 61 patients with chronic systolic HF [52].

<table>
<thead>
<tr>
<th>Study (reference)</th>
<th>Study population, n (Mean age, years)</th>
<th>Specimen and method</th>
<th>Results</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Woitas et al. [44]</td>
<td>Angiographically confirmed CAD, 2997 (63)</td>
<td>Plasma, particle-enhanced turbidimetric immunoassay</td>
<td>The risk of all-cause- and CV death was highest in 4th quartile compared to 3rd tertile (reference) when adjusted for conventional cardiovascular risk factors, except when adjusting for creatinine.</td>
<td>Plasma NGAL levels reflect the degree of inflammatory processes in patients with CAD.</td>
</tr>
<tr>
<td>Kalkas et al. [45]</td>
<td>Angiographically confirmed CAD, 35 (64.5)</td>
<td>Serum ELISA</td>
<td>Significant differences in serum NGAL levels were seen between stable CAD patients and unstable CAD - NSTEMI – and STEMI patients.</td>
<td>Serum NGAL levels reflect the degree of inflammatory processes in patients with CAD.</td>
</tr>
</tbody>
</table>

**Table 2 (continued)**

<table>
<thead>
<tr>
<th>Study population, n (Mean age, years)</th>
<th>Specimen and method</th>
<th>Results</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stable CAD = 1408 Unstable CAD = 951 Controls = 638</td>
<td>Plasma, particle-enhanced turbidimetric immunoassay</td>
<td>The risk of all-cause- and CV death was highest in 4th quartile compared to 3rd tertile (reference) when adjusted for conventional cardiovascular risk factors, except when adjusting for creatinine.</td>
<td>Plasma NGAL levels reflect the degree of inflammatory processes in patients with CAD.</td>
</tr>
<tr>
<td>Stable CAD = 40 (63.8) Unstable CAD = 35 (64.6) NSTEMI = 40 (64.5) STEMI = 25 (64.2) Healthy controls = 20 (41.5)</td>
<td>Serum ELISA</td>
<td>Significant differences in serum NGAL levels were seen between stable CAD patients and unstable CAD - NSTEMI – and STEMI patients.</td>
<td>Serum NGAL levels reflect the degree of inflammatory processes in patients with CAD.</td>
</tr>
</tbody>
</table>

**Table 2**
Table 3: Neutrophil gelatinase associated-lipocalin studies on cardiac surgery and AKI included in this review.

<table>
<thead>
<tr>
<th>Study (reference)</th>
<th>Study population, n (Mean age, years)</th>
<th>Specimen and method</th>
<th>Results</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mishra et al. [16]</td>
<td>Children undergoing cardiac surgery, 71 (3)</td>
<td>Urine and serum Western blot and ELISA</td>
<td>Significant correlation between AKI and serum/urinary NGAL 2 h postoperatively. AUC: 0.998/0.906.</td>
<td>Strong correlation between serum/urinary NGAL and AKI after cardiac surgery.</td>
</tr>
<tr>
<td>Dent et al. [48]</td>
<td>Children undergoing cardiac surgery, 120 (4)</td>
<td>Plasma Triage (point-of-care) NGAL device</td>
<td>Plasma NGAL levels increased significantly within 2 h. AUC: 0.96 for prediction of AKI.</td>
<td>Plasma NGAL strongly correlated with duration of AKI and was an early predictor of AKI after paediatric CPB.</td>
</tr>
<tr>
<td>Xin et al. [47]</td>
<td>Adults undergoing cardiac surgery, 33 (38)</td>
<td>Urine and serum ELISA</td>
<td>Urine NGAL levels peaked within 2–4 h. AUC: 0.926.</td>
<td>Urinary NGAL corrected for creatinine was a useful biomarker in detection of AKI after cardiac surgery.</td>
</tr>
<tr>
<td>Tuladhar et al. [17]</td>
<td>Adults undergoing cardiac surgery, 50 (67)</td>
<td>Urine and plasma ELISA</td>
<td>High plasma/urinary NGAL levels in patients who developed AKI. AUC: 0.96/0.80 for the detection of post-CPB renal dysfunction.</td>
<td>No significant difference in serum NGAL levels in patients with and without AKI. Urinary/plasma NGAL was an early biomarker in detection of AKI in patients undergoing cardiac surgery.</td>
</tr>
<tr>
<td>Friedrich et al. [49]</td>
<td>Adults undergoing cardiac surgery, 81 (67)</td>
<td>Urine CMIA</td>
<td>Urinary NGAL increased during cardiac surgery (after 120 min) and postoperatively, but did not correlate with postoperative kidney injury.</td>
<td>Urinary NGAL did not predict acute postoperative kidney injury.</td>
</tr>
</tbody>
</table>

EUSA, enzyme-linked immunosorbent assay; AKI, acute kidney injury; CPB, cardiopulmonary bypass; AUC, area under curve; CMIA, Chemilumineszenz-Micropartikelimmunoassay.
### Table 4: Neutrophil gelatinase associated-lipocalin studies on patients with heart failure.

<table>
<thead>
<tr>
<th>Study (reference)</th>
<th>Study population, n (Mean age, years)</th>
<th>Specimen and method</th>
<th>Results</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yndestad et al. [6]</td>
<td>Adults with CHF, 150 (56)</td>
<td>Serum ELISA</td>
<td>Higher NGAL levels in NYHA III/IV vs. NYHA I/II and controls.</td>
<td>Patients with high serum NGAL levels had a higher incidence of the composite endpoint of non-fatal MI, CV death, total mortality death and stroke.</td>
</tr>
<tr>
<td>Bolignano et al. [51]</td>
<td>Elderly with CHF, 46 (78)</td>
<td>Plasma ELISA</td>
<td>Higher NGAL levels in NYHA IV vs. NYHA I/II/III and controls AUC: 0.68. Hazard ratio for death (cut-off NGAL &gt;783 ng/mL) 6.08.</td>
<td>Plasma NGAL levels were strongly correlated with NYHA class.</td>
</tr>
<tr>
<td>Villacorta et al. [52]</td>
<td>Adults with CHF, 61 (61.6)</td>
<td>Plasma Triage (point-of-care) NGAL test</td>
<td>NGAL cutoff to predict events 179 ng/mL. AUC: 0.73. Patients with primary endpoint had higher NGAL levels at baseline.</td>
<td>Patients above the NGAL cutoff had a shorter event-free survival.</td>
</tr>
<tr>
<td>Damman et al. [53]</td>
<td>Adults with CHF, 90 (59)</td>
<td>Urine ELISA</td>
<td>Urinary NGAL levels were increased in patients vs. healthy controls.</td>
<td>Renal impairment (tubular damage) in CHF patients may be measured by increased urinary NGAL concentration.</td>
</tr>
<tr>
<td>Nymo et al. [54]</td>
<td>Adults with CHF, 1415 (72)</td>
<td>Serum ELISA</td>
<td>After adjusting for ApoA-1, eGFR, hsCRP and NTproBNP, NGAL did not remain a significant predictor for the endpoints.</td>
<td>NGAL added no significant information to NT-proBNP for primary and secondary endpoints. However, NGAL provided information about adverse endpoints, when only adjusting for demographic and clinical variables.</td>
</tr>
<tr>
<td>Maisel et al. [55]</td>
<td>Adults diagnosed with AHF, 186 (70)</td>
<td>Plasma Triage (point-of-care) NGAL device</td>
<td>Patients with events had higher NGAL levels than patients without events. AUC: 0.72.</td>
<td>NGAL was a prognostic indicator of 30 day outcomes in patients admitted for AHF.</td>
</tr>
</tbody>
</table>

ELISA, enzyme-linked immunosorbent assay; AUC, area under curve; NYHA, New York Heart Association; eGFR, estimated glomerular filtration rate; hs-CRP, high-sensitivity C-reactive protein; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; AHF, acute heart failure; CHF, chronic heart failure; MI, myocardial infarction; CV, cardiovascular.
The primary endpoint was a composite of cardiovascular death and admission for heart failure during 10.6 months (median) follow up. In 15 patients who reached the endpoint, higher baseline NGAL concentrations were observed. The study concluded that plasma NGAL may be a predictor of outcome in patients with HF. Furthermore, the study reported a positive correlation between plasma NGAL and creatinine, yet no association was observed between plasma NGAL and BNP. This finding is in agreement with Lindberg et al. who did not report any association between NGAL and NT-proBNP [56].

In chronic HF patients, urine NGAL levels at admission were associated with increased risk of subsequent worsening of renal function [53]. Another study reported that urinary NGAL was associated with a combined primary endpoint of all-cause mortality and HF hospitalisation in chronic HF patients [57]. Both studies indicated that urinary NGAL may be a direct marker of renal damage and tubulo-interstitial injury in chronic HF patients.

Nymo et al. [54] studied the prognostic value of serum NGAL in elderly with chronic ischaemic HF. When adjusted for demographic and clinical variables, NGAL concentration remained a significant predictor of adverse outcome. However, when adjusted for apolipoprotein A-1 (ApoA-1), estimated glomerular filtration rate (eGFR), hs-CRP, and NT-proBNP, this association was lost. The authors concluded that the clinical use of circulating NGAL in this study was limited.

Maisel et al. [55] investigated the prognostic value of plasma NGAL in patients with acute HF. Patients were followed for 30 days, and the primary combined endpoint was HF readmission or all-cause mortality. Baseline and discharge levels of NGAL were significantly elevated in patients with events compared to event-free patients. Patients with events also had higher BNP levels. The AUC by using ROC analysis was higher for NGAL [0.73] than for BNP [0.65], indicating that plasma NGAL is a good predictor of the combined endpoint after 30 days in acute HF patients. In addition, the combination of high-NGAL and high-BNP or high-NGAL and low-BNP was associated with higher risk of HF readmission or all-cause death than low-NGAL and low-BNP or low-NGAL and high-BNP.

Both plasma and urinary NGAL levels are increased in chronic HF patients. The elevation is dependent on the clinical stage of HF and the presence of renal failure. Plasma NGAL may amplify the predictive value of NT-proBNP and increase the risk of adverse outcome. Thus, the combined elevation of NGAL and NT-proBNP is associated with the worst outcome.

Overall, NGAL demonstrated a questionable discrimination between categories and disease severity of CVD. Discrepancies are also seen between urine and plasma NGAL, where the performance of urine NGAL in most studies is superior to plasma for determining AKI. These differences may be due to different origins of NGAL in urine and plasma in AKI and CVD. During AKI, NGAL is primarily secreted from the kidney, whereas the pool of NGAL in blood may originate from several sources including neutrophils during inflammation, which may contribute to the observed differences in performance of urine and plasma NGAL in AKI and CVD [58, 59].

Future perspectives

Based on the aforementioned studies, the potential clinical use of circulating NGAL in cardiac settings remains to be defined. Several factors may contribute to the diversity in NGAL levels and ultimately its association to endpoints. In the studies reviewed above, the heterogeneous subpopulations, lack of appropriate reference material for assay calibration, and potential differences in assay specificity towards the monomeric, dimeric, and heterodimeric forms of NGAL make direct comparisons challenging. While most studies measured NGAL levels at baseline, ideally, repetitive measurements of NGAL levels may enable monitoring of disease progression. Furthermore, this approach could provide additional information on the correlation with other inflammatory biomarkers and may also enhance the predictive value of NGAL. However, source and timing of blood sampling are still an issue in many studies investigating NGAL and CVD, since NGAL is most likely secreted at the site of active inflammation. Duration of CVD prior to study inclusion may also influence NGAL levels.

Further studies establishing standardised reference ranges for NGAL taking gender, age, and ethnicity into consideration are also warranted. Future research should also evaluate whether combinations of NGAL and cardiac biomarkers such as troponin and BNP provide stronger associations with clinical outcomes than conventional biomarkers. Future studies should specifically explore if incorporating NGAL into clinical care may benefit patient management.

Another aspect is to consider co-existing diseases such as malignancy, chronic kidney disease, and systemic inflammatory conditions, which may affect systemic NGAL levels and interfere with the association of NGAL
and CVD. In this case, collection of data about whether patients were exposed to nephrotoxic agents is important.

**Summary and conclusions**

Despite preventive and therapeutic efforts, morbidity and mortality due to CVD remains an increasing challenge. Therefore, new biomarkers are warranted to identify CVD patients with an increased risk of cardiovascular events, which may guide therapeutic strategies to improve prognosis. NGAL has emerged as a promising biomarker of outcomes in CVD.

In previous studies, NGAL has performed as a more consistent predictor of adverse outcomes in patients with ACS, especially STEMI, compared with patients having stable CAD. This may indicate that NGAL is mainly secreted at active inflammatory sites, and is suggested as a complementary risk marker to NT-Pro-BNP and hs-CRP. These biomarkers along with NGAL may allow better prediction of outcomes in CAD patients.

NGAL may be a risk marker of AKI after cardiac surgery. The diagnostic/prognostic accuracy of NGAL in both AKI and CVD is varying in different studies illustrated by non-standardised reference ranges, measurement methods, different specimen types, anticoagulants, storage, and stability conditions, cohort size, clinical settings, and subpopulations. There is, however, ample evidence to support the use of urine NGAL in AKI [60]. While urine NGAL in AKI almost exclusively contain NGAL monomer from the kidney, the plasma/serum pool contain different NGAL complexes from several sources and would therefore be expected to show poorer diagnostic performance in AKI.

Although data suggest a potential role for NGAL as a biomarker in HF patients, further studies are needed to evaluate the prognostic relevance of this biomarker, as the majority of the aforementioned studies included only small patient groups.

Overall, the studies showed that urinary NGAL is primarily elevated in patients with AKI, while plasma/serum NGAL is significantly increased in patients with acute MI as well as stable CAD. Conversely, plasma NGAL still reflects AKI when the renal clearance of NGAL is decreased. In order to demonstrate associations between NGAL and outcomes independent of AKI, adjustments for kidney function, e.g. creatinine and/or eGFR or parallel measurements of NGAL in both urine and plasma are needed. However, a uniform AKI definition and timing of NGAL measurements in research studies are still needed.

Future studies may explore new therapeutic strategies using tailored treatment based on measurements of NGAL. Moreover, further studies should explore whether NGAL is a strong and specific marker of atherosclerosis.

**Author contributions:** All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

**Research funding:** None declared.

**Employment or leadership:** None declared.

**Competing interests:** The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

**References**


