

Neutrophil Gelatinase Associated Lipocalin (NGAL) as a Biomarker. Does It Apply in Abdominal Aortic Aneurysms? A Review of Literature

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Abstract Neutrophil gelatinase associated lipocalin (NGAL) as a protein derived from neutrophils has recently been the field of investigation in a wide range of diseases (renal disease, coronary artery disease, etc). The MEDLINE/PubMed database was searched for publications with the medical subject heading “NGAL” and keywords “Abdominal aortic aneurysm (AAA),” “biomarker,” and “growth”. We restricted our search to date. In this review, we included 38 articles and abstracts that were accessible and available in English. An effort to further explain the role of NGAL within AAA has been made. NGAL seems to be a hopeful marker for the pathogenesis and the progression of abdominal aortic aneurysms (AAAs), which has significant morbidity and mortality rates.

Keywords Neutrophil gelatinase associated lipocalin (NGAL) · Abdominal aortic aneurysm (AAA) · Biomarkers · Growth

Introduction

Abdominal aortic aneurysm (AAA) originating from the Greek word *ανεύρυσμα*, which stands for a dilatation or widening of the abdominal aorta. The most accepted

definition of an AAA is based on the diameter of the abdominal aorta: an abdominal aortic diameter of 3.0 cm or more, which is usually more than two standard deviations above the mean diameter for both men and women [1–3]. Other authors have defined AAA as the maximum infrarenal aortic diameter being at least 1.5 times larger than the expected normal to compensate for individual variation in the diameter of the adjacent aorta [4–6]. AAA disease may become lethal if left untreated [7], leading to 2–3 % of all deaths in the elderly [8]. Its pathogenesis is multifactorial involving extracellular matrix breakdown, smooth muscle cell disappearance, and inflammation. Better understanding of molecular mechanisms is an important step to clarify the pathophysiology and role of genetic and molecular biomarkers as well as developing new therapeutic strategies for AAA. Unfortunately, there are no specific laboratory markers that would allow one to distinguish in a simple way between aneurysm bearers and the healthy population. This is the reason why the development and progression of AAAs have been linked to various inflammatory mediators, among which stands neutrophil gelatinase associated lipocalin (NGAL) [9].

In this article, we aim to give a conceptual description of the potential role of NGAL in predicting the natural history of AAA. This review article could become an eye-opener for future studies in AAA, since at present no markers as such are available to predict the rupture risk.

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Methods

The MEDLINE/PubMed database was searched for publications with the medical subject heading “NGAL” and keywords “Abdominal aortic aneurysm (AAA),” “biomarker,” and “growth”. We restricted our search to date.

Results

In this review, we included 38 articles and abstracts that were accessible and available in English. An effort to further explain the role of NGAL within AAA has been made.

Nature of NGAL

NGAL was originally identified as a 25-kDa protein, covalently bound to matrix metalloproteinase-9 (MMP-9) from neutrophils [10]. Mature peripheral neutrophils lack NGAL-mRNA expression, and NGAL protein is synthesized at the early myelocyte stage of granulopoiesis during formation of secondary granules [11]. Like other lipocalins, NGAL forms a barrel-shaped tertiary structure with a hydrophobic calyx that binds small bacterial catecholate-type ferric lipophilic molecules [12, 13]. The major ligands for NGAL are siderophores, which are small iron-binding molecules that are synthesized by bacteria to acquire iron from the surroundings. NGAL depletes siderophores and it could therefore be hypothesized that NGAL participates in the antibacterial iron depletion strategy of the innate immune system [14]. NGAL is normally expressed in human neutrophils and in other cell types such as epithelial, renal tubular, and hepatic cells during inflammation or injury [15].

Interestingly, NGAL is induced in a number of human cancers and is often a marker of poor prognosis. A variety of tumor-promoting agents, such as SV40, polyoma virus, hepatocyte growth factor, and glucocorticoids, induce the gene of NGAL [16]. The methods identifying NGAL levels are Western blot, ELISA, and determination of cell-free DNA (cf-DNA) [17]. ELISA currently available, such as Bioporto and R&D Systems, are accurate but still not practical in the clinical setting.

Plasma NGAL concentrations have been associated with cardiovascular risk in patients with asymptomatic atherosclerosis [18]. Among patients with coronary artery disease (CAD) and chronic heart failure (CHF), serum levels of NGAL increase proportionally with the severity of disease [19]. NGAL plasma concentration has been correlated with renal function markers (creatinine) in patients with carotid atherosclerosis [20]. Additionally, a progressive and significant increase occurs in serum NGAL following acute cerebrovascular events (ischemic stroke) and persists for up to 1 year [21, 22].

NGAL and AAA Pathogenesis

How does NGAL come up in the pathogenesis of AAA? The answer to this question involves three points. Firstly, cells within the intraluminal thrombus (ILT) produce large amounts of neutrophil activators like myeloperoxidase (MPO), plasmin-antiplasmin complexes (PAP), and D-dimers (DD).

These markers activate and provoke the chemotaxis of neutrophils, which are the main source of NGAL [17, 23]. Neutrophil may contribute to two main mechanisms of AAA evolution, namely, medial destruction and adventitial immune-inflammatory processes [9]. Depletion of neutrophils is able to inhibit experimental AAA formation [24]. Secondly, through the junction with MMP-9, the degradation of the latter is inhibited, thereby preserving enzymatic activity. In this way, further proteolytic degradation of elastin takes place, thus leading to the growth of the AAA [25]. Upregulation of both MMP-9 and NGAL is induced by c-Jun N-terminal-kinase (JNK), which is involved in cellular stress responses and has been shown to be an important signaling molecule in the pathogenesis of AAA [26]. Besides the above, NGAL possesses bacteriostatic properties by binding bacterial siderophores, in this way preventing bacteria from retrieving iron sources [27]. This suggests that the release of NGAL from the neutrophils in the iron-rich ILT, functions as a proteolytic response to increased bacteria present in AAA tissue [28].

Experimental evidence for this role is derived from genetically modified mice that lack NGAL gene, which makes them more susceptible to Gram-negative bacterial infections and death from sepsis [29]. Interestingly, *Chlamydia pneumoniae*-reactive T lymphocytes have been identified in AAA [14], and chlamydia has been localized to AAA tissue [30]. However, whether the increased expression of NGAL in the thrombus is a result of bacterial infection is not known.

The development of AAA is linked to degradation of elastin and collagen. These changes are more pronounced in the aneurysm wall covered by the ILT, which also shows more signs of inflammation and is thinner compared to the aneurysm wall exposed to flowing blood [31]. The rate of increase in diameter of AAA correlates with increased thrombus growth and rupture [31].

NGAL and AAA Natural History

Several studies have taken place in order to investigate the role of NGAL in AAA patients. Ramos-Mozo et al. [17] found that neutrophils (PMNs) isolated from AAA patients secreted significantly greater amounts of NGAL than from controls (115 ng/ml (78–200) vs. 94 ng/ml (72–114) $p < 0.001$). Luminal thrombus released large amounts of NGAL compared to abluminal AAA thrombus, AAA wall, and healthy aortic media [17]. NGAL plasma concentrations in AAA patients were also correlated with other markers of thrombus activity (PAP and DD) [17]. Furthermore, a positive correlation between plasma NGAL and retrospective AAA growth ($p = 0.01$) was observed [17].

Studies using gene array of human aneurysm specimens have shown that most MMPs were upregulated in the thrombus-free wall [31]. Analyses by zymography, however,

demonstrate gelatinase activity in the interface between the thrombus and the underlying wall and in the media of the wall not covered by a thrombus [31]. The thrombus contains large amounts of neutrophils [31]. Thus, NGAL is involved in the regulation of MMP-9 activity and prevents its inactivation, thus augmenting the proteolytic effect [31]. The presence of NGAL/MMP-9 complexes throughout the thrombus and in the thrombus-covered wall may also contribute to the increased proteolytic degradation seen in this wall segment [31].

A study of mediators of neutrophil recruitment in human abdominal aortic aneurysms driven by Houard et al [23] found that NGAL/MMP-9 (cutoff level of 5 µg) complexes as soluble neutrophil marker were found in supernatants of the luminal ILT layer with a significant negative gradient from the luminal to the abluminal poles of the ILT ($p < 0.01$). In addition, the release of NGAL/MMP-9 was found in higher amounts in AAA patients compared to healthy controls ($p < 0.02$) [23]. Additionally, it was also concluded that NGAL plasma levels as a neutrophil marker did not correlate with either maximal aortic diameter or maximal ILT thickness ($p = 0.8$ and 0.2 , respectively) [32].

Finally, Folkesson et al. [25] came to the conclusion that the concentration of the NGAL/MMP-9 complex was highest in the luminal part of the thrombus. Neutrophil-derived NGAL could enhance the proteolytic activity associated with AAA, but the importance of this mechanism for aneurysm growth remains to be shown [25].

As far as the intra- and postoperative values of NGAL are concerned, AAA endovascular repair induces their increase ($182 \pm 115\%$ $p = 0.008$) [7]. In cases of open AAA repair, it is shown that after clamp removal, NGAL washes out from the lower extremities, which become ischemic during aneurysm repair [33]. Additionally, AAA surgery triggers an inflammatory reaction and thus the increase of NGAL, which is known to be an acute-phase reactant produced by some immune cells [33]. NGAL has further been widely studied as an individual marker of acute kidney injury (AKI) induced by the AAA

repair surgery, and it has been shown to increase due to decreased GFR with subsequent clearance impairment per se [34, 35].

More specifically, as far as open AAA repair and NGAL level changes are concerned, an increase in serum NGAL (75.21 ± 55.83 vs. 46.37 ± 21.60 ng/ml, $p > 0.05$) and significantly elevated plasma NGAL levels at 2 h (91.54 ± 76.54 vs. baseline, $p < 0.05$), 12 h (100.78 ± 44.92 vs. baseline, $p < 0.05$), and 24 h (89.46 ± 94.18 vs. baseline, $p < 0.05$) after clamp release and reperfusion were found [33, 34].

As far as urinary NGAL is concerned, there was a significant elevation at 12 h (20.75 ng/ml [5.00 – 176.10] vs. 5.85 ng/ml [1.40 – 16.00] at baseline, $p < 0.05$) and 24 h (13.95 [3.90 – 163.30] vs. baseline, $p < 0.05$) after clamp release [34, 35].

Speelman et al [36] showed that ILT causes a reduction in wall stress, which was stronger for larger thrombi and higher elastic moduli ($p < 0.01$). A larger ILT was associated with a higher AAA growth rate, but also with a lower wall stress [36]. Therefore, weakening of the AAA wall, under the influence of thrombus, may play a more imminent role in the process of AAA growth than the stress acting on the wall.

Furthermore, low AAA wall stress was associated with a lower aneurysm growth rate [37]. Growth rate was also positively related to MMP-9 plasma concentration ($r = 0.32$) [37]. The average MMP-9 and CRP concentrations increased with increasing degrees of relative wall stress, although the absolute and relative wall stress did not correlate with any of the biomarkers [37].

All in all, after meticulous analysis of literature to identify an established correlation between NGAL levels and AAA wall stress, we failed to find a direct correlation between them. Although both ILT and MMP-9 seem to be related with AAA wall stress. So, further studies are needed to shed light to this potential relationship.

Table 1 summarizes the role of NGAL in the natural history of AAA.

Table 1 The role of NGAL in the natural history of AAA

Author	Year	Study	Results
Ramos-Mozo [17]	2012	Increased plasma levels of NGAL, a marker of neutrophil activation, in patients with AAA	<ul style="list-style-type: none"> Releases from PMNs and by the luminal part of AAA thrombus could be a surrogate marker of the thrombus biological activity is increased in AAA patients ($p < 0.001$) correlated with retrospective AAA growth ($p < 0.01$)
Houard [23, 32]	2009	Mediators of NGAL, neutrophil recruitment in human AAA	<ul style="list-style-type: none"> In complex with MMP-9 is found in higher amounts in AAA patients does not correlate with either maximal aortic diameter or maximal ILT thickness ($p = 0.8$ and 0.2 respectively)
Folkesson [25]	2007	Presence of NGAL/MMP-9 complexes in human AAA	<ul style="list-style-type: none"> NGAL/MMP-9 complexes is present in AAA and thrombus NGAL could enhance the proteolytic activity associated with AAA
Swedenborg [31]	2006	ILT as a source of proteolytic activity.	<ul style="list-style-type: none"> The bind between NGAL and MMP-9 may lead to further degradation of AAA wall and increase the risk of rupture

Conclusions

All in all, NGAL is shown to be released by resident and circulating leucocytes of AAA patients, thereby playing the role of a marker of the size and progression of the AAA. Besides, it seems to be present in patients with angiopathy in general (in cases of renal angiopathy, coronary artery disease), suggesting in this way a possible use as a prognostic and/or predictive marker in such diseases. In the field of AAA, where a few substances can play such a role, NGAL appears to be a hopeful biomarker on which further investigation should be done. As far as its correlation with AAA wall stress, more studies aiming to highlight this potential relationship are mandatory.

Conflict of Interest The authors have no conflict of interest.

References

- Steinberg I, Stein HL (1966) Arteriosclerotic abdominal aortic aneurysms. Report of 200 consecutive cases diagnosed by intravenous aortography. *JAMA* 195:1025
- McGregor JC, Pollock JG, Anton HC (1975) The value of ultrasonography in the diagnosis of abdominal aortic aneurysm. *Scott Med J* 20:133–137
- Wanhainen A, Thermo R, Ahlström H, Lind L, Johansson L (2008) Thoracic and abdominal aortic dimension in 70-years old men and women—a population-based whole-body MRI study. *J Vasc Surg* 47:504–512
- Sterpetti A, Schultz R, Feldhaus R, Cheng S, Peetz D (1987) Factors influencing enlargement rate of small abdominal aortic aneurysms. *J Surg Res* 43:211–219
- Collin J, Walton J, Araujo L, Lindsell D (1988) Oxford screening programme for abdominal aortic aneurysm in men aged 65 to 74 years. *Lancet* 2:613–615
- Sonesson B, Lanne T, Hansen F, Sandgren T (1994) Infrarenal aortic diameter in the healthy person. *Eur J Vasc Surg* 8:89–95
- Chang CK, Chuter TA, Niemann CU, Shlipak MG, Cohen MJ, Reilly LM et al (2009) Systemic inflammation, coagulopathy, and acute renal insufficiency following endovascular thoracoabdominal aortic aneurysm repair. *J Vasc Surg* 49(5):1140–1146
- Sakalihan N, Limet R, Defawe OD (2005) Abdominal aortic aneurysm. *Lancet* 365(9470):1577–1589
- Michel JB, Thauant O, Houard X, Meilhac O, Caligiuri G, Nicoletti A (2007) Topological determinants and consequences of adventitial responses to arterial wall injury. *Arterioscler Thromb Vasc Biol* 27(6):1259–1268
- Devarajan P (2007) Emerging biomarkers of acute kidney injury. *Contrib Nephrol* 156:203–212
- Devarajan P (2010) Neutrophil gelatinase-associated lipocalin: a promising biomarker for human acute kidney injury. *Biomark Med* 4(2):265–280
- Akerstrom B, Flower DR, Salier JP (2000) Lipocalins: unity in diversity. *Biochim Biophys Acta* 1482:1–8
- Goetz DH, Holmes MA, Borregaard N et al (2002) The neutrophil lipocalin NGAL is a bacteriostatic agent that interferes with siderophore-mediated iron acquisition. *Mol Cell* 10:1033–1043
- Kol A, Sukhova GK, Lichtman AH et al (1998) Chlamydial-heat shock protein 60 localizes in human atheroma and regulates macrophage tumor necrosis factor- α and matrix metalloproteinase expression. *Circulation* 98:300–307
- Kjeldsen L, Cowland JB, Borregaard N (2000) Human neutrophil gelatinase-associated lipocalin and homologous proteins in rat and mouse. *Biochim Biophys Acta* 1482(1–2):272–283
- Devarajan P (2010) The promise of biomarkers for personalized renal cancer care. *Kidney Int* 77(9):755–757
- Ramos-Mozo P, Madrigal-Matute J, Vega de Ceniga M, Blanco-Colio LM, Meilhac O, Feldman L et al (2012) Increased plasma levels of NGAL, a marker of neutrophil activation, in patients with abdominal aortic aneurysm. *Atherosclerosis* 220(2):552–556
- Elneihoum AM, Falke P, Hedblad B, Lindgärde F, Ohlsson K (1997) Leukocyte activation in atherosclerosis: correlation with risk factors. *Atherosclerosis* 131(1):79–84
- Yndestad A, Landrø L, Ueland T, Dahl CP, Flo TH, Vinge LE et al (2009) Increased systemic and myocardial expression of neutrophil gelatinase-associated lipocalin in clinical and experimental heart failure. *Eur Heart J* 30(10):1229–1236
- Giaginis C, Zira A, Katsargyris A, Klonaris C, Theocharis S (2010) Clinical implication of plasma neutrophil gelatinase-associated lipocalin (NGAL) concentrations in patients with advanced carotid atherosclerosis. *Clin Chem Lab Med* 48(7):1035–1041
- Elneihoum AM, Falke P, Axelsson L, Lundberg E, Lindgärde F, Ohlsson K (1996) Leukocyte activation detected by increased plasma levels of inflammatory mediators in patients with ischemic cerebrovascular diseases. *Stroke* 27(10):1734–1738
- Anwaar I, Gottsäter A, Ohlsson K, Mattiasson I, Lindgärde F (1998) Increasing levels of leukocyte-derived inflammatory mediators in plasma and cAMP in platelets during follow-up after acute cerebral ischemia. *Cerebrovasc Dis* 8(6):310–317
- Houard X, Ollivier V, Louedec L, Michel JB, Bäck M (2009) Differential inflammatory activity across human abdominal aortic aneurysms reveals neutrophil-derived leukotriene B₄ as a major chemotactic factor released from the intraluminal thrombus. *FASEB J* 23(5):1376–1383
- Eliason JL, Hannawa KK, Ailawadi G, Sinha I, Ford JW, Deogracias MP et al (2005) Neutrophil depletion inhibits experimental abdominal aortic aneurysm formation. *Circulation* 112(2):232–240
- Folkesson M, Kazi M, Zhu C, Silveira A, Hemdahl AL, Hamsten A et al (2007) Presence of NGAL/MMP-9 complexes in human abdominal aortic aneurysms. *Thromb Haemost* 98(2):427–433
- Yoshimura K, Aoki H, Ikeda Y, Furutani A, Hamano K, Matsuzaki M (2006) Regression of abdominal aortic aneurysm by inhibition of c-Jun N-terminal kinase in mice. *Ann N Y Acad Sci* 1085:74–81
- Flo TH, Smith KD, Sato S, Rodriguez DJ, Holmes MA, Strong RK et al (2004) Lipocalin 2 mediates an innate immune response to bacterial infection by sequestering iron. *Nature* 432(7019):917–921
- Delbosc S, Alsac JM, Journe C, Louedec L, Castier Y, Bonnefont-Mallet M et al (2011) *Porphyromonas gingivalis* participates in pathogenesis of human abdominal aortic aneurysm by neutrophil activation. Proof of concept in rats. *PLoS One* 6(4):e18679
- Berger T, Togawa A, Duncan GS et al (2006) Lipocalin 2-deficient mice exhibit increased sensitivity to *Escherichia coli* infection but not to ischemia-reperfusion injury. *Proc Natl Acad Sci U S A* 103:1834–1839
- Juvonen T, Biancari F, Juvonen J (2002) Chlamydia pneumoniae and aortic aneurysms. *Scand Cardiovasc J* 36:327–328
- Swedenborg J, Eriksson P (2006) The intraluminal thrombus as a source of proteolytic activity. *Ann N Y Acad Sci* 1085:133–138
- Houard X, Touat Z, Ollivier V, Louedec L, Philippe M, Sebbag U et al (2009) Mediators of neutrophil recruitment in human abdominal aortic aneurysms. *Cardiovasc Res* 82(3):532–541

33. Grigoryev DN, Liu M, Hassoun HT et al (2008) The local and systemic inflammatory transcriptome after acute kidney injury. *J Am Soc Nephrol* 19:547–558
34. Kokot M, Biolik G, Ziaja D et al (2012) Acute kidney injury after abdominal aortic aneurysm surgery: detailed assessment of early effects using novel markers. *Pol Arch Med Wewn* 122(7–8):353–360
35. Lacquaniti A, Giardina M, Lucisano S, Messina R, Buemi A, Risitano CD et al (2014) Neutrophil gelatinase-associated lipocalin (NGAL) and endothelial progenitor cells (EPCS) evaluation in aortic aneurysm repair. *Curr Vasc Pharmacol* 11(6):1001–1010
36. Speelman L, Schurink GW, Bosboom EM, Buth J, Breeuwer M, van de Vosse FN, Jacobs MH (2010) The mechanical role of thrombus on the growth rate of an abdominal aortic aneurysm. *J Vasc Surg* 51(1):19–26
37. Speelman L, Hellenthal FA, Pulinx B, Bosboom EM, Breeuwer M, van Sambeek MR et al (2010) The influence of wall stress on AAA growth and biomarkers. *Eur J Vasc Endovasc Surg* 39(4):410–416