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Original Article

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Neutrophil Gelatinase-Associated Lipocalin: A New Biomarker for the Differential Diagnosis of Anemia?

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Abstract

Objective: Differential diagnosis of iron deficiency anemia (IDA) and anemia of chronic disease (ACD) is performed based on some biochemical indicators of iron metabolism. Although these traditional indicators are useful to determine iron status, they may not provide conclusive criteria for determining iron deficiency because of their high variability and low sensitivity. The aim of the present study was to evaluate the clinical significance of neutrophil gelatinase-associated lipocalin (NGAL) in the differential diagnosis of IDA and ACD and to compare it with other conventional anemia parameters.
Materials and Methods: A total of 35 patients with IDA with serum hemoglobin<12 g/dL, transferrin saturation (TSAT)<20%, and serum ferritin<20 ng/mL and 31 patients with ACD with serum hemoglobin<12 g/dL, TSAT >20%, and serum ferritin >50 ng/mL were enrolled in the study.
Results: The median serum NGAL values were 0.44 (0.32-0.66) ng/mL in the IDA group and 2.16 (1.39-3.22) ng/mL in the ACD group, and a statistically significant difference was observed between the two groups with respect to NGAL (p<0.001). NGAL showed a significant positive correlation with hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, serum iron, TSAT, and ferritin (p<0.001) and a significant negative correlation with transferrin and total iron-binding capacity (p<0.001). The NGAL cut-off value was obtained as 1.02 ng/mL (97.1% sensitivity and 83.9% specificity) for the differentiation of IDA from ACD by receiver operating characteristic analysis.
Conclusion: Our data suggest that NGAL can be a useful parameter in the differential diagnosis of IDA and ACD. An NGAL cut-off value of 1.02 ng/mL predicts differential diagnosis.
Keywords: Anemia, diagnosis, NGAL

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INTRODUCTION

Iron deficiency anemia (IDA) and anemia of chronic disease (ACD) are the most prevalent forms of anemia encountered in general practice (1). Differentiation between IDA and ACD is based on a standard panel consisting of ferritin, serum iron, total iron-binding capacity (TIBC) or transferrin, and transferrin saturation (TSAT) (2-4). Despite the availability of these parameters, their validity for the diagnosis of IDA is still debatable. Serum ferritin, the most specific indicator of iron deficiency, is an acute-phase reactant, and its levels are affected by inflammation. TSAT fluctuates depending on the diurnal variation of serum iron. While serum iron levels decrease with infection, inflammation, and malignancy, its concentrations increase with liver disease (5).

Neutrophil gelatinase-associated lipocalin (NGAL), a small 25 kDa peptide identified as a component of neutrophil granules, inhibits bacterial growth by depleting their intracellular iron stores (6). NGAL is also a promising marker of kidney injury that predicts acute renal impairment (7). Overexpression of NGAL is associated with a poor prognosis in a variety of cancers including breast carcinomas (8). In addition, NGAL was shown to induce

apoptosis of primary bone marrow cells including erythroid progenitor cells and inhibit erythroid cell production leading to anemia (9-12). Finally, NGAL has been proposed as a new tool in the assessment of iron deficiency and in the management of iron therapy for hemodialysis patients (13).

The aim of the present study was to evaluate the significance of NGAL as a biomarker for the differential diagnosis of IDA and ACD and to compare its effectiveness with other conventional anemia parameters.

MATERIALS AND METHODS

Patients

The study was conducted at Eskişehir Osmangazi University, Faculty of Medicine, Department of Hematology. After obtaining the approval of the ethics committee and informed consent, 35 patients with IDA (serum hemoglobin<12 g/dL, TSAT<20%, and serum ferritin<20 ng/mL) and 31 patients with ACD (serum hemoglobin<12 g/dL, TSAT >20%, and serum ferritin >50 ng/mL) were enrolled in the study. Patients with a history of polycystic kidney disease and kidney transplantation and patients with hemodialysis or peritoneal dialysis were excluded from the study. All the subjects with ACD were patients with chronic kidney disease under follow-up of the Nephrology Department of our hospital who have an estimated glomerular filtration rate value of between 15 and 60 mL/min/1.73 m^2 according to the Chronic Kidney Disease Epidemiology Collaboration formula.

Sample Collection and Laboratory Methods

Samples collected in K3EDTA tubes for complete blood count were analyzed on an automated hematology analyzer (ADVIA 2120i; Siemens, NY, USA). Serum iron and TIBC were measured by the LISA 500 Plus automated chemical analyzer (Hycel Diagnostics, Paris, France). Serum ferritin was measured on the Hitachi E170 automated analyzer (Hitachi, Tokyo, Japan). TSAT was calculated as TSAT=(Fe/TIBC) × 100. Transferrin was measured by the BN II automated chemical analyzer (Siemens, Marburg, Germany). NGAL was measured using the ELISA commercially available kit (R&D Systems, MN, USA).

Statistical Analysis

Data were analyzed using IBM Statistical Package for the Social Sciences (IBM SPSS Statistics Corp.; Armonk, NY, USA) version 20 and MedCalc version 12.7.4.0. Unpaired *t*-test was applied for normally distributed values, and results were expressed as mean ± standard deviation. Mann–Whitney U test was utilized for non-normally distributed values, and results were expressed as median (interquartile range) values. The Spearman correlation coefficient was used to test the correlations between NGAL and other variables considered in the study. Receiver operating characteristic (ROC) analysis was em-

Table 1. Differences in laboratory parameters between patients with IDA and patients with ACD

	IDA (n=35) Median (IQ range)	ACD (n=31) Median (IQ range)	p
Hb (g/dL)	10.4 (8.9-11.6)	10.3 (9.2-11.1)	0.452
Htc (%)*	29.7±3.7	32.1±3.9	<0.001
MCV (fL)	72.5 (66.9-78.2)	86.4 (82.7-88.9)	<0.001
White blood cell (10^3/µL)	7.9 (6.3-10.1)	6.6 (5.3-8.6)	0.029
Platelet*	271 (203-349)	232 (196-283)	0.175
MCH (pg)	23.2 (20.7-26.4)	29.8 (27.9-31.1)	<0.001
MCHC (g/dL)	31.9 (30.8-33.2)	34.3 (33.6-35.4)	<0.001
Iron (mg/dL)	29.0 (21.0-37.0)	47.0 (32.0-72.0)	0.001
TIBC (mg/dL)	356.0 (324.0-396.0)	213.0 (170.0-234.0)	<0.001
% Sat	8.0 (6.3-13.3)	28.2 (18.5-43.6)	<0.001
Transferrin (mg/dL)	280.0 (239.0-309.0)	176.0 (143.0-209.0)	<0.001
Creatinine (mg/dL)	0.7 (0.6–0.9)	7.4 (6.2–8.5)	<0.001
Ferritin (ng/mL)	5.3 (3.2–9.5)	226.0 (133.4–440.2)	<0.001
NGAL (ng/mL)	0.44 (0.32–0.68)	2.16 (1.39–3.22)	<0.001

*Data are expressed as mean±standard deviation.
IDA, iron deficiency anemia; ACD, anemia of chronic disease; IQ, interquartile; Hb, hemoglobin; Htc, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; TIBC, total iron-binding capacity; % Sat, % saturation of transferrin; NGAL, neutrophil gelatinase-associated lipocalin

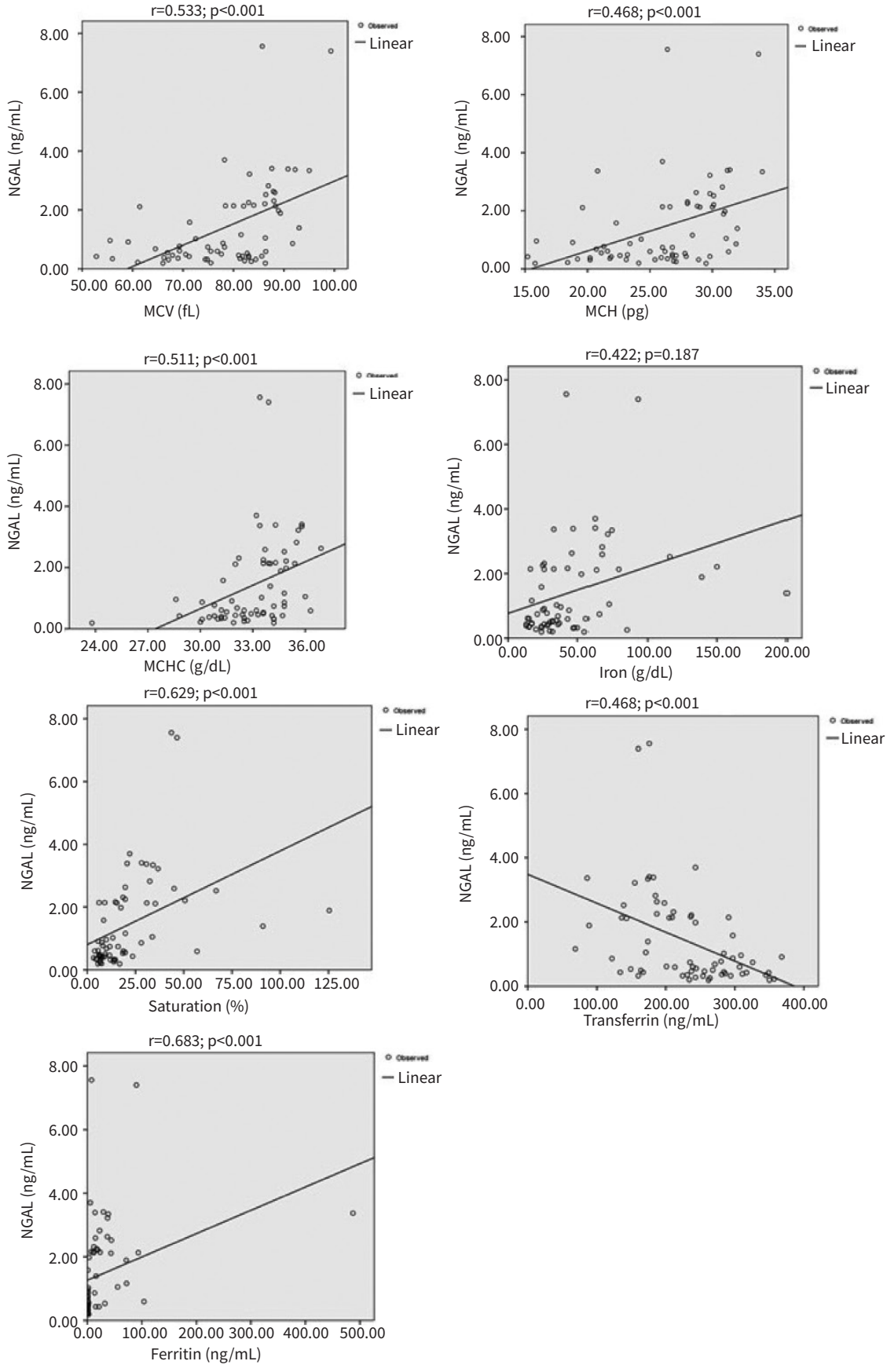

Figure 1. Correlations between conventional hematological parameters and NGAL

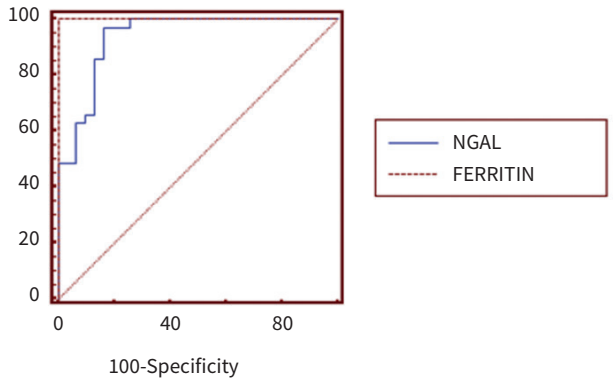

Figure 2. Receiver operating characteristics (ROC) curves of NGAL and serum ferritin

ployed to calculate the area under the curve (AUC) for NGAL and serum ferritin and to find the best NGAL cut-off value for predicting the differentiation of IDA from ACD. A p-value<0.05 was considered significant.

RESULTS

The present measurements showed that hematocrit (29.7% vs. 32.1%), mean corpuscular volume (MCV) (72.5 fl vs. 86.40 fl), mean corpuscular hemoglobin (MCH) (23.2 pg vs. 29.8 pg), mean corpuscular hemoglobin concentration (MCHC) (31.9 g/dL vs. 34.3 g/dL), serum iron (29.0 mg/dL vs. 47.0 mg/dL), ferritin (5.3 ng/mL vs. 226.0 ng/mL), and TSAT (8.0% vs. 28.2%) levels were significantly lower in patients with IDA than in those with ACD. Transferrin (280 mg/dL vs. 176 mg/dL) and TIBC (356 mg/dL vs. 213 mg/dL) levels were markedly higher in patients with IDA than in those with ACD. Patients with IDA presented with lower serum NGAL values (median 0.44 ng/mL) than those with ACD (median 2.16 ng/mL); the difference in the levels of NGAL was significant between the two groups (p<0.001) (Table 1).

The correlations between conventional hematological parameters and NGAL were also evaluated. NGAL showed a significant (p<0.001) positive correlation with MCV (r=0.533), MCH (r=0.468), MCHC (r=0.511), serum iron (r=0.422), TSAT (r=0.629), and ferritin (r=0.683), whereas it showed a significant (p<0.001) negative correlation with transferrin (r=0.468) and TIBC (r=0.559) (Figure 1).

ROC analysis was performed to assess the diagnostic potential of NGAL in the differential diagnosis of IDA versus ACD. AUC for NGAL was 0.936 (95% confidence interval (CI) 0.848-0.982, p<0.0001). The best NGAL cut-off value was determined as 1.02 ng/mL (97.1% sensitivity and 83.9% specificity) (Figure 2). Power analysis of NGAL was calculated as 1, which was perfect (NCCS 2007, PASS 2005, and GESS 2006). ROC curves of NGAL and ferritin considering IDA as a status variable are shown in Figure 3. AUCs for NGAL and ferritin were 0.936 (95% CI 0.848–0.982) and 1.000 (95% CI 0.946–1.000), respectively; these areas were significantly different (p=0.0322).

ROC curves of NGAL and serum ferritin considering iron deficiency (TSAT<20%) as a status variable were employed to assess the diagnostic potential of NGAL in the differential diagnosis of IDA versus ACD and to compare NGAL with serum ferritin in discriminating these types of anemia. AUCs for NGAL and serum ferritin were 0.936 (95% CI 0.848-0.982) and 1.000 (95% CI 0.946-1.000), respectively; these areas were slightly different (p=0.032). The best NGAL cut-off value was ≤1.02 ng/mL with a sensitivity of 97.14% (95% CI 85.1-99.9) and a specificity of 83.87% (95% CI 66.3-94.5).

DISCUSSION

Clinically, IDA and ACD are the most common forms of anemia encountered in general practice. Differentiation between IDA and ACD is based on a standard panel (serum iron, TIBC, TSAT, and ferritin). Although these traditional parameters are useful to determine iron status, they may not provide conclusive criteria for determining iron deficiency because of their high variability and low sensitivity. For example, ferritin acts as an acute-phase reactant that limits its diagnostic accuracy greatly. The serum ferritin level is frequently increased independent of iron status by many factors, such as acute/chronic inflammation, infection, malignancy, liver disease, and alcohol use. Serum iron levels also decrease with infection, inflammation, and malignancy and increase with liver disease. TSAT is a calculated parameter, thereby reflecting confounding effects on individual components (1-5). In addition to the current conventional parameters that we use in routine practice to diagnose IDA, there is still a need for more sensitive and powerful parameters. We, therefore, conducted this prospective study to evaluate the significance of NGAL as a biomarker for the differential diagnosis of IDA and ACD and to compare its effectiveness with other conventional anemia parameters.

NGAL, expressed by neutrophils, is a true acute-phase protein, originally discovered as an antibacterial factor in natural immunity. This small 25 kDa peptide of the lipocalin superfamily has recently been shown to bind small, iron-carrying molecules called siderophores (14, 15). In a recent murine model, serum lipocalin-2 (Lcn2) protein levels were shown to be markedly increased after different experimental models of anemia induced by phlebotomy, iron deprivation, phenylhydrazine administration, or hypoxia and suggested a possible physiological role for Lcn2 during increased iron utilization and mobilization from its stores (16). Weizer-Stern et al. (17) reported a decreased expression of hepcidin along with increased NGAL expression in the liver in beta-thalassemia mouse models.

Lcn2 is expressed abundantly in erythroid progenitor cells. An in vitro culture experiment demonstrated that Lcn2 induces apoptosis and inhibits the differentiation of erythroid progenitor cells (12). During a condition of primary anemia, Bolignano et al. (15) explained a double response with respect to NGAL production and its systemic effects. As NGAL is an antioxidant factor, there is an increase in the peripheral production of the

peptide to counteract hypoxic stress. However, a reduction in the production of NGAL is indicated since increased NGAL production would have a negative effect on bone marrow erythroid cells. Hence, several systemic diseases associated with secondary anemia, such as chronic renal failure, cancer, and inflammation, are known to induce an increase in circulating NGAL values. Anemia of chronic inflammation might be partly due to increased levels of Lcn2 secreted from expanded leukocytes and/or macrophages, and anemia of cancer might be partly due to the plentiful secretion of Lcn2 from tumor cells (12). In our study, patients with ACD presented significantly higher serum NGAL values than those with IDA. Our finding may represent an important cause of the development and worsening of anemia and suggests that inhibition of Lcn2 may constitute an effective therapy for anemia under certain pathological conditions.

Recently it has been reported that NGAL might be proposed as a new tool in the assessment of iron deficiency and in the management of iron therapy for hemodialysis patients (13). With the present investigation, we identified the value of NGAL in the differential diagnosis of IDA and ACD that are the most prevalent forms of anemia encountered in general practice. NGAL showed a significant positive correlation with hematocrit, MCV, MCH, MCHC, serum iron, TSAT, and ferritin and a significant negative correlation with transferrin and TIBC. In our study, NGAL also showed a good diagnostic power in identifying IDA, with the best cut-off value of ≤1.02 ng/mL, as determined by ROC analysis. Since AUC for NGAL was quite close to AUC for ferritin, it is concluded that NGAL can be used as a new biomarker for the differential diagnosis of anemia, whereas its diagnostic ability should be improved further by studies including larger sample sizes. Although the results were so encouraging, we believe that a new study design with a higher number of patients could give more accurate and realistic cut-off value for NGAL.

We also did not include patients with chronic renal failure with IDA in the present study. This is because the main reason of our study was to evaluate the clinical significance of NGAL in the differential diagnosis of IDA and ACD. A group of patients with both clinical situations would be confusing, but as a limitation, this kind of patients could be included as a third group into the study.

A major limitation of the present study is that we did not compare NGAL with other new diagnostic tools used in the differential diagnosis of IDA versus ACD, such as soluble transferrin receptor (sTfR) or sTfR/ferritin index.

CONCLUSION

The present data suggest that NGAL can be used as a reliable differentiating marker in the diagnosis of IDA and ACD, in addition to the current conventional parameters that we use in routine practice, for the differential diagnosis of anemia. Although the results were so encouraging, we believe that a new study design with a higher number of patients could give more accurate and realistic cut-off value for NGAL.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Eskişehir Osmangazi University.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept G.T.; Design – G.T., M.A.; Supervision - G.T., M.A.; Resources – G.T., M.A.; Materials – M.K.; Data Collection and/or Processing – G.T., M.A., M.K., S.T.; Analysis and/or Interpretation – G.T., M.A., F.M.; Literature Search – M.A., M.K., S.T., A.U.Y.; Writing Manuscript – G.T, M.K.; Critical Review – M.A., A.U.Y.

Conflict of Interest: The authors have no conflicts of interest to declare.

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REFERENCES

1. Weiss G, Goodnough LT. Anemia of chronic disease. N Engl J Med 2005; 352: 1011-23. [CrossRef]
2. Ferguson BJ, Skikne BS, Simpson KM, Baynes RD, Cook JD. Serum transferrin receptor distinguishes the anemia of chronic disease from iron deficiency anemia. J Lab Clin Med 1992; 119: 385-90.
3. Ganz T. Anemia of chronic disease. In Williams Hematology. Eds.: Lichtman MA, Beutler E, Kipps TJ, Seligsohn U, Kaushansky K, Prchal JT, New York: McGraw-Hill 2006; 7th ed., 565-70.
4. Baer AN, Dessypris EN, Krantz SB. The pathogenesis of anemia in rheumatoid arthritis: A clinical and laboratory analysis. Semin Arthritis Rheum 1990; 19: 209-23. [CrossRef]
5. Fishbane S, Galgano C, Langley RC Jr, Canfield W, Maesaka JK. Reticulocyte hemoglobin content in the evaluation of iron status of haemodialysis patients. Kidney Int 1997; 52: 217-22. [CrossRef]
6. Goetz DH, Holmes MA, Borregaard N, Bluhm ME, Raymond KN, Strong RK. The neutrophil lipocalin NGAL is a bacteriostatic agent that interferes with siderophore-mediated iron acquisition. Mol Cell 2002; 10: 1033-43. [CrossRef]
7. Bolignano D, Donato V, Coppolino G, Campo S, Buemi A, Lacquaniti A, et al. Neutrophil gelatinase-associated lipocalin (NGAL) as a marker of kidney damage. Am J Kidney Dis 2008; 52: 595-605. [CrossRef]
8. Bauer M, Eickhoff JC, Gould MN, Mundhenke C, Maass N, Friedl A. Neutrophil gelatinase-associated lipocalin (NGAL) is a predictor of poor prognosis in human primary breast cancer. Breast Cancer Res Treat 2008; 108: 389-97. [CrossRef]
9. Devireddy LR, Teodoro JG, Richard FA, Green MR. Induction of apoptosis by a secreted lipocalin that is transcriptionally regulated by IL-3 deprivation. Science 2001; 293: 829-34. [CrossRef]
10. Lin H, Monaco G, Sun T, Ling X, Stephens C, Xie S, et al. Bcr-Abl-mediated suppression of normal hematopoiesis in leukemia. Oncogene 2005; 24: 3246-56. [CrossRef]
11. Miharada K, Hiroyama T, Sudo K, Danjo I, Nagasawa T, Nakamura Y. Lipocalin 2-mediated growth suppression is evident in human erythroid and monocyte/macrophage lineage cells. J Cell Physiol 2008; 215: 526-37. [CrossRef]
12. Miharada K, Hiroyama T, Sudo K, Nagasawa T, Nakamura Y. Lipocalin 2 functions as a negative regulator of red blood cell production in an autocrine fashion. FASEB J 2005; 19: 1881-3. [CrossRef]
13. Bolignano D, Coppolino G, Romeo A, De Paola L, Buemi A, Lacquaniti A, et al. Neutrophil gelatinase-associated lipocalin (NGAL)

reflects iron status in haemodialysis patients. Nephrol Dial Transplant 2009; 24: 3398-403. [CrossRef]
14. Xu S, Venge P. Lipocalins as biochemical markers of disease. Biochim Biophys Acta 2000; 1482: 298-307. [CrossRef]
15. Bolignano D, Coppolino G, Donato V, Lacquaniti A, Bono C, Buemi M. Neutrophil gelatinase-associated lipocalin (NGAL): A new piece of the anemia puzzle? Med Sci Monit 2010; 16: 131-5.
16. Jiang W, Constante M, Santos MM. Anemia upregulates lipocalin 2 in the liver and serum. Blood Cells Mol Dis 2008; 41: 169-74. [CrossRef]
17. Weizer-Stern O, Adamsky K, Amariglio N, Rachmilewitz E, Breda L, Rivella S, et al. mRNA expression of iron regulatory genes in beta-thalassemia intermedia and beta-thalassemia major mouse models. Am J Hematol 2006; 81: 479-83. [CrossRef]