# Evaluation of Serum and Urinary Neutrophil Gelatinase Associated Lipocalin (NGAL) in Children with Nephrotic Syndrome

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## Abstract

**Background:** Nephrotic syndrome in children is a common disease in the world, and is a group of clinical symptoms and include (loss of protein in the urine, low protein level in the blood, the accumulation of fluid in the body or edema, and high blood lipids). The cause of Nephrotic Syndrome is unknown. Neutrophil gelatinase associated lipocalin is a protein encoded in humans by the LCN2 gene. NGAL interferes with natural immunity by blocking iron, causing the growth of germs to be reduced. It is expressed in kidney, prostate, respiratory and digestive cells. NGAL is used as a vital guide to kidney injury.

**Method:** Neutrophil gelatinase-associated lipocalin concentration was measured in blood plasma using enzyme-linked immunosorbent assay (ELISA) and urine NGAL by Abbott i1000, measured plasma and urine level of NGAL in 20 patients with steroid resistance nephrotic syndrome,20 patients with steroid sensitive nephrotic syndrome, 17 patients with early diagnosed nephrotic syndrome compare with 20 healthy volunteers enlisted as normal controls.

**Results:** Urine NGAL and plasma NGAL are significantly increase in SRNS, SSNS, EDNS compared with control group.

**Conclusion:** NGAL can be used to diagnostic biomarker to predict steroid Sensitive or steroid resistance in children with Nephrotic syndrome (NGAL is a differentiating marker between SSNS and SRNS).

***Keywords:*** *Nephrotic Syndrome; Lipocalin; Neutrophil Gelatinase; serum.*

## Introduction

Nephrotic syndrome is an edema, proteinuria, hypoalbuminaemia, and hyperlipidemia clinical constellation. The main pathology of this clinical syndrome is enhanced permeability of the glomerular filtration barrier to proteins (Sampson et al 2015). nephrotic range proteinuria is defined as proteinuria more than 1000 mg/m$^2$ per 24 hours or spot urinary protein/ creatinine ratio more than 2 mg/mg. the proteinuria in children NS is relatively selective, constituted mainly by albumin [(1)] .NS is collection of symptoms including heavy proteinuria (protein/creatinine ratio >2mg/mg), hypoalbuminaemia (<2.5mg/L), hypercholesterolemia and general edema. This resulting from altered permeability of the glomerular basement membranes[(2)]. Nephrotic syndrome (NS) is an alteration in renal function leads to increased permeability to plasma protein like albumin in the glomerular basement membrane. Edema, marked or persistent proteinuria and hypoalbuminaemia include signs and symptoms. There were three types of NS identified: primary NS (MCNS), secondary NS and

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congenital NS. NS were found. NS is the most prevalent form of minimal change NS, it represents 80 percent of instances and happens at any era; it is most prevalent in men[3]. NGAL is protein of the lipocalin superfamily. NGAL) also known to as lipocalin-2, siderocalin, uterocalin, and 24p3 is a polypeptide .NGAL is a critical component of innate immunity to bacterial infection and is expressed by immune cells, hepatocytes, and renal tubular cells in various disease states.

## Materials and Method

**Study design case control study:** The present study include 77 Iraqi participants (20 with SRNS, 20 with SSNS,17 with EDNS,20 normal healthy control group) the Age range (3-13)years, the age and gender matched to the patients and control group. Blood sample and Urine collected from February 2019 to June 2019. The following biochemical parameters have been studied.

**NGAL ELISA:** ELISA kit uses the principle Sandwich-ELISA. The micro-plate ELISA providing in ELISA kit pre covered with an antibody specific to Human NGAL. Standards or samples are added to the micro-plate ELISA wells and combine with the specific antibody. And then a biotinylated detection antibody specific for Human NGAL and Avidin Horseradish Peroxidase (HRP) conjugate are added consecutively to each micro-plate well and incubation. Wash away the free components. The substrate solution is added to each well in micro-plate ELISA. Only those wells that contain Human NGAL, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometric at a wavelength of 450 nm . The OD value is proportional to the concentration of Human NGAL .Calculation of the concentration of Human NGAL in the samples compared to the OD of the samples to the standard curve.

**Statistical Analysis:** Statistical analysis was carried out by using SPSS version 23 and Microsoft excel 2010. the numerical data expressed as mean ± SD. Comparison between mean plasma and urineNGAL concentration groups were performed. Receiver Operating Characteristics (ROC) Curve was calculated to estimate the sensitivity and specificity of the used NGAL.

## Results

There was highly significant difference in mean NGAL among study groups ($P < 0.0001$); the level was highest in SRNS, SSNS, and EDNS patients with highly significant difference ($P < 0.0001$) in comparison with control subjects

**Table (1): Mean and standard deviation for urine and plasma NGAL**

| **Parameters** | **Controls (N=20) (mean±SD)** | **SRNS(N=20) (mean±SD)** | **P-value** |
|---|---|---|---|
| Urine NGAL | 64.7±19.8 | 174.3±18.4 | <0.0001 |
| Plasma NGAL | 2.19±0.92 | 11.4±1.4 | <0.0001 |
| **Parameters** | **Controls (N=20) (mean±SD)** | **SSNS (N=20) (mean±SD))** | **P-value** |
| Urine NGAL | 64.7±19.8 | 80.9±9.0 | 0.002 |
| Plasma NGAL | 2.19±0.92 | 5.5±1.14 | <0.0001 |
| **Parameters** | **Controls (N=20) (mean±SD))** | **EDNS (N=17) (mean±SD)** | **P-value** |
| Urine NGAL | 64.7±19.8 | 128.4±41.1 | <0.0001 |
| Plasma NGAL | 2.19±0.92 | 9.3±3.4 | <0.0001 |
| **Parameters** | **SRNS(N=20) (mean±SD)** | **SSNS (N=20) (mean±SD)** | **P-value** |
| Urine NGAL | 174.3±18.4 | 80.9±9.0 | <0.0001 |
| Plasma NGAL | 11.4±1.4 | 5.5±1.14 | <0.0001 |
| **Parameters** | **SSNS(N=20) (mean±SD)** | **EDNS(N=17) (mean±SD)** | **P-value** |
| Urine NGAL | 80.9±9.0 | 128.4±41.1 | <0.0001 |
| Plasma NGAL | 5.5±1.14 | 9.3±3.4 | <0.0001 |

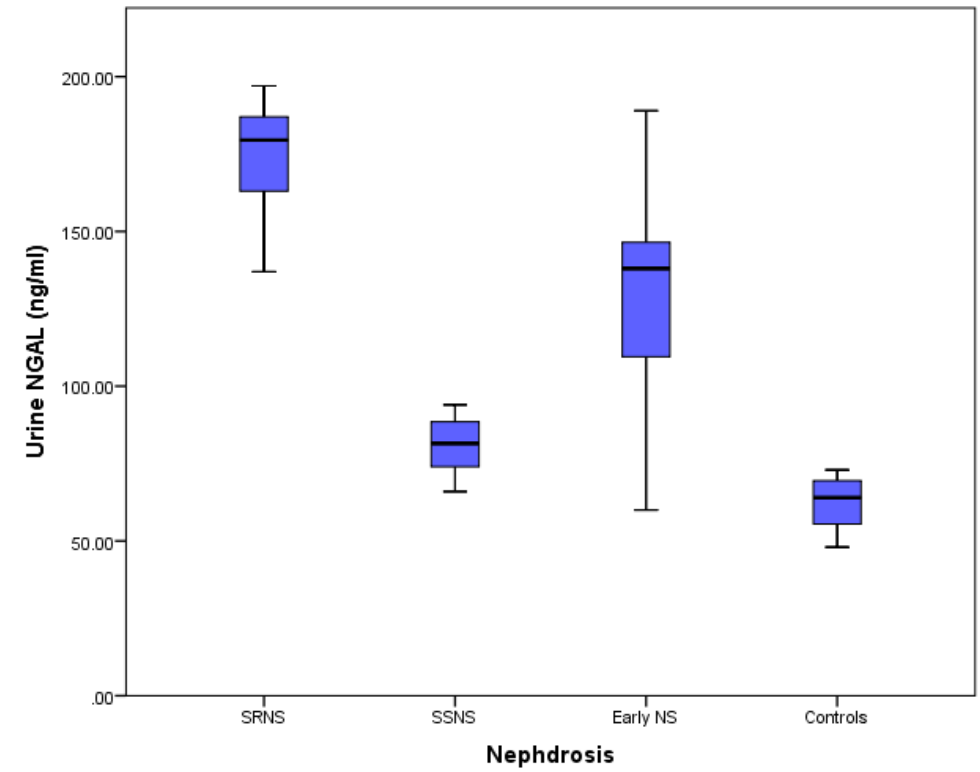


**Figure (1): urine NGAL distribution among childrenwith NS and controls**

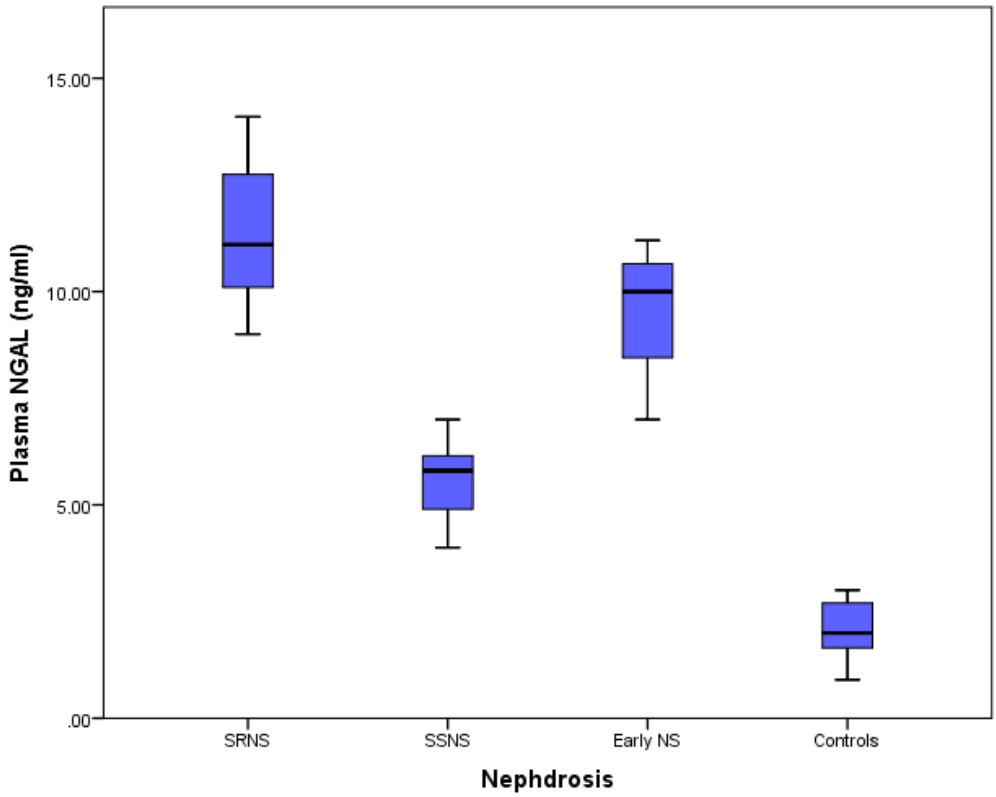


**Figure (2): Plasma NGAL distribution among childrenwith NS and controls**

**Determination the expected value (cutoff value) of NGAL in urine of children with Nephrotic syndrome:** The Receiver Operator Characteristic (ROC) curve shows a significant differentiated ability of elevation urine NGAL as shown in figure (3).

The cutoff value of urine NGAL concentration in SRNS was 137 ng/ml with sensitivity 95% and specificity 95%, whereascutoff value of urine NGAL concentration in SSNS was75 ng/ml with sensitivity 75% and specificity 90%,whereascutoff value of urineNGAL concentration in EDNS was90 ng/ml with sensitivity 85% and specificity 95%.

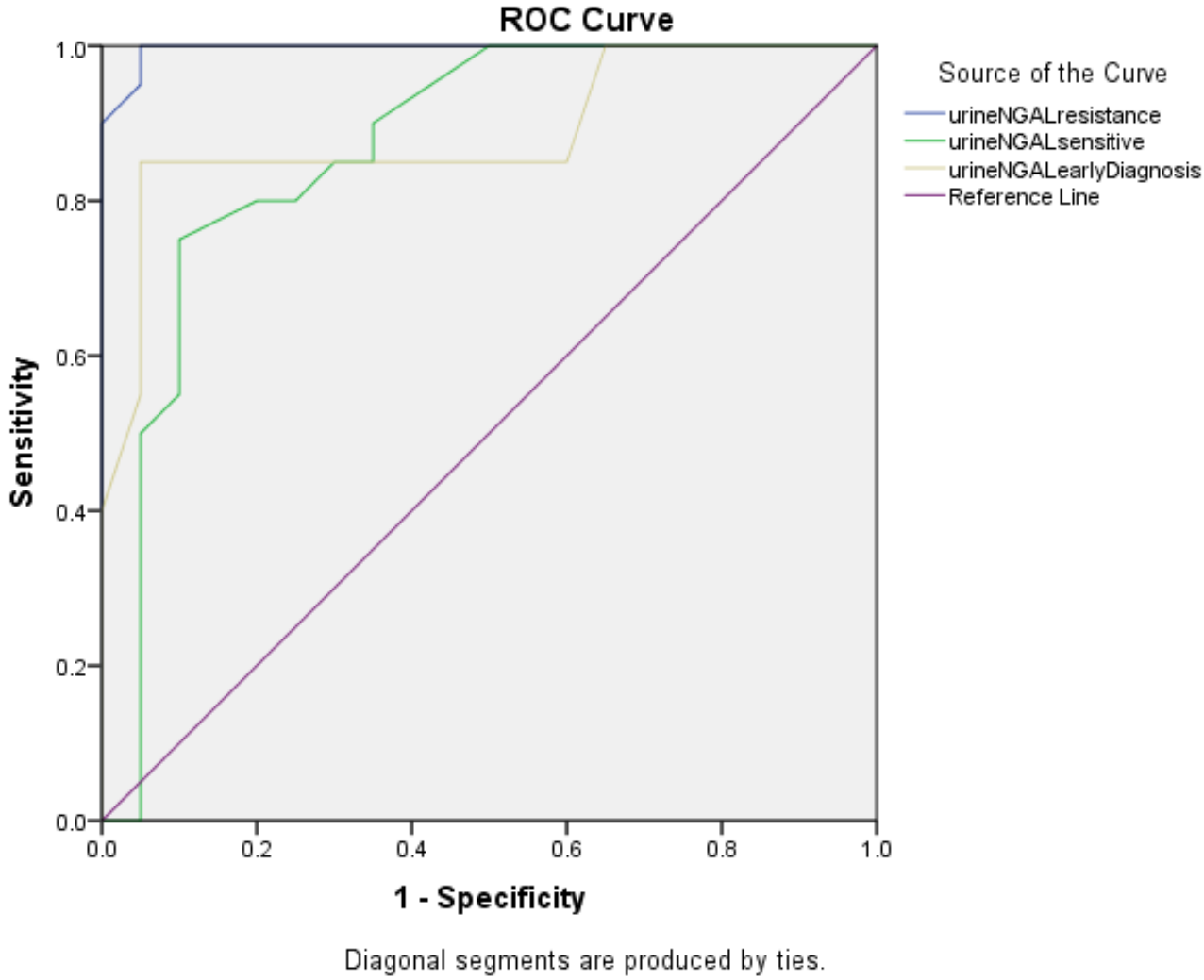


**Figure (3): ROC curve of NGAL (ng/ml) in urine of nephrotic syndrome children compared with control subject**

**Determination the expected value (cutoff value) of NGAL in plasma of children with Nephrotic syndrome:** The Receiver Operator Characteristic (ROC) curve shows a significant differentiated ability of elevation plasmaNGAL as shown in figure (4).

The cutoff value of the plasmaNGAL concentration in SRNS was 4.0 ng/ml with sensitivity 95% and specificity 90%,whereas the cutoff value of plasmaNGAL concentration in SSNSwas 3.5 ng/ml with sensitivity 95% and specificity 90%, Whereas the cutoff value of plasmaNGAL concentration in EDNS was6.0 ng/ml with sensitivity 85% and specificity 95% .

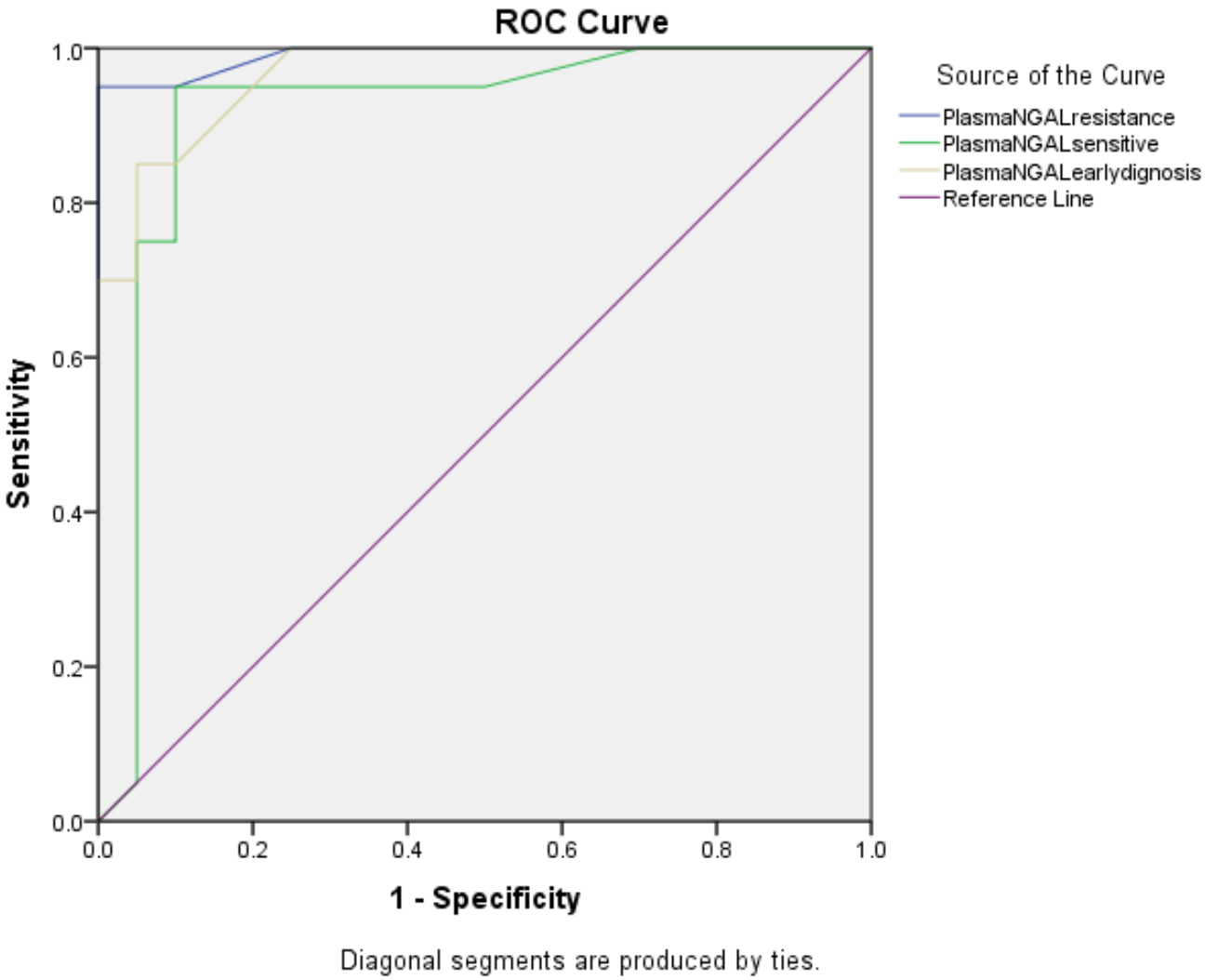


**Figure (4): ROC curve of NGAL (ng/ml) in plasmaof nephrotic syndrome children compared with control subject**

## Discussion

The present study showed the mean ± SD of NGAL in urine of SRNS, SSNS, EDNS and controls groups were (174.3±18.4), (80.9±9.0), (124.1±43.6) and (56.5±12.3). respectively and showed elevated of urine NGAL in SRNS,SSNS and EDNS compare with control group,

And the mean ± SD of NGAL in plasma of SRNS, SSNS, EDNS and controls groups were (11.4±1.4), (5.5±1.1), (9.3±3.4) and (2.19±0.92) respectively and showed elevated of NGAL in SRNS, SSNS and EDNS compare with control group.

Secreted NGAL in the distal nephron in nephrosis especially in chronic state (SRNS) lead to continuous release of NGAL in blood and excreted in urine while in SSNS the release of NGAL is through periods not continuous.This explains difference in concentrations between groups of nephrotic syndrome. In early diagnosed nephrotic syndrome amount of NGAL begin release relation to severity state,this agree with [4].

Other studyBolignano and Buemi 2009 explain the effects of Cyclosporine treatment (immunosuppressant drugs) in SRNS can cause nephrotoxicity due to progressive glomerular vasoconstriction leads increase urine NGAL concentrations.

Correlations among NGAL concentrations and proteinuria concentration and also between plasma NGAL and urine NGAL values were noted[5],[6]. However, although possible, this model does not exclude that the same tubular cells can contribute equally considerably to

the massive elevation of urine NGAL, at least in part: from this point of perspective, at least two mechanisms could be possible. Firstly, the aforementioned cubilin-megalin carrier, responsible for NGAL resorption, acts through a mechanism of nonspecific protein endocytosis that recognizes several other serum ligands such as albumin, beta2-microglobulin, and serum immunoglobulin. In confirmation of this, murine models of knockout mice for megalin soon develop a condition of severe nonselective proteinuria, even in the absence of documentable glomerular histological lesions[7]. Under conditions of sustained proteinuria (e.g., nephrotic syndrome), this nonspecific carrier soon becomes saturated because of the massive tubular protein overload, causing further loss of plasma proteins that in part contributes to determine the extent of final proteinuria[8]. It is also to be considered that the main site of the damage induced by proteinuria through complement activation is precisely the brush border of the tubular cells, where the most of the cubilin-megalin complexes are located: this condition would further contribute to compromise NGAL endocytosis by its carrier. Ultimately, it cannot be excluded, however, that the same tubular cells, subjected to stress from the insult caused by the activity of complement factors, actively produce and release high amounts of NGAL with a defensive significance similar to what observed in experimental models of acute kidney damage. In accordance with this, previous studies have shown that the tubular epithelium responds to a sustained load of plasma proteins through the release of multiple "stress proteins" including KIM-1, whose urinary levels accordingly rise in a dramatic way [9].

## Conclusions

Neutrophil Gelatinase Associated Lipocalin can be used to diagnostic biomarker to predict steroid Sensitive or steroid resistance in children with Nephrotic syndrome (NGAL is a differentiating marker between SSNS and SRNS).

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest:** The authors declare that they have no conflict of interest.

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