Research Article

A Road To Molecular Disease Of Childhood—Sickle Cell Disease Cases Series

Bhavya Dharshini D[1], M Preethi[2]

[1]Bhavya Dharshini D, 1st-year postgraduate student, Department of Pathology, Sree Balaji Medical College and Hospital, Chennai, Tamil Nadu., Mail ID: drbhavyadharshini4@gmail.com , ORCID ID: https://orcid.org/0009-0009-1483-1140
[2]M Preethi, Associate Professor, Department of Pathology, Sree Balaji Medical College and Hospital, Chennai, Tamil Nadu., Mail ID: drpreethi1290@gmail.com, ORCID ID: https://orcid.org/0000-0001-9851-1455
Corresponding author: M.Preethi

Abstract:

Sickle cell disease (SCD) is a hereditary hemoglobin disorder commonly presenting during childhood. It results from a point mutation in the β-globin gene, leading to the substitution of a single amino acid within the hemoglobin molecule. Inheritance follows an autosomal recessive pattern. According to estimates from the World Health Organization, hemoglobinopathies affect nearly 15% of the global population. The worldwide incidence of homozygous SCD is approximately 170 cases per 100,000 live births, with the highest prevalence observed in Africa, where the incidence reaches about 1,125 cases per 100,000 live births.

The present study describes a series of pediatric and adolescent patients diagnosed with sickle cell disorders, focusing on their clinical presentation, associated complications, and treatment strategies. Fetal hemoglobin (HbF), which consists of two alpha and two gamma globin chains, differs structurally from adult hemoglobin (HbA), composed of two alpha and two beta globin chains. HbF plays a protective role by reducing red blood cell sickling under stressful physiological conditions. Because HbF levels remain relatively high during early infancy and gradually decline after birth, symptoms of SCD generally become evident after six months of age. In settings where neonatal screening programs are unavailable, diagnosis is frequently delayed until clinical manifestations appear.

This study highlights the clinicopathological spectrum of sickle cell disease and emphasizes current diagnostic and management approaches for affected patients.

Keywords: Sickle cell disease (SCD); autosomal; Haemoglobin.

Introduction:

Sickle cell disease (SCD) is a genetically inherited disorder characterized by chronic hemolytic anemia resulting from the production of abnormal hemoglobin. Hemoglobin, the oxygen-carrying component of red blood cells, plays a vital role in delivering oxygen from the lungs to various tissues of the body. While normal erythrocytes contain hemoglobin A (HbA), individuals with SCD possess abnormal hemoglobin variants, particularly hemoglobin S (HbS), and in some cases hemoglobin C (HbC)[1].

Healthy red blood cells exhibit a flexible biconcave disc shape that allows them to traverse the microvasculature efficiently. The underlying molecular defect in SCD is a point mutation in the β-globin gene, causing the amino acid valine to replace glutamic acid at the sixth position of the β-globin chain [2]. This seemingly minor alteration significantly changes the physicochemical properties of hemoglobin, leading to red blood cell deformation and sickling under deoxygenated conditions.

The homozygous inheritance of the HbS gene results in sickle cell anemia (HbSS), the most severe form of the disease. Individuals carrying one normal β-globin allele and one HbS allele are described as having sickle cell trait (HbAS) and are generally asymptomatic carriers. The spectrum of sickle cell disease also includes compound heterozygous conditions such as hemoglobin SC disease (HbSC), sickle β^+-thalassemia (HbS/β^+), and sickle β^o-thalassemia (HbS/β^o). These disorders arise when the HbS mutation is inherited together with another abnormal hemoglobin or thalassemia allele [3,4]

Although substantial improvements in medical care have enhanced patient outcomes, sickle cell disease continues to be associated with significant morbidity and premature mortality. Reported average survival ranges from approximately 30 to 45 years, with life expectancy influenced by disease-related complications and the availability of appropriate healthcare services.

Case report no. 1

An 8-year-old male child presented to the Surgical Outpatient Department with a painful ulcer over the leg that

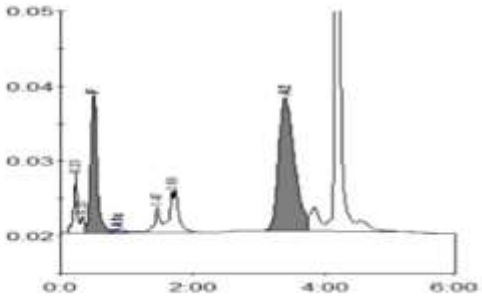
had persisted for approximately 18 days. The patient also experienced recurrent episodes of musculoskeletal pain, abdominal discomfort, and intermittent fever. He had undergone cholecystectomy recently for gallstone disease. His past medical history was notable for hip surgery performed during infancy. There was no history of jaundice, chronic respiratory disease, or other significant comorbid conditions.

On clinical examination, the child was markedly pale, and mild splenomegaly was detected. Laboratory investigations revealed severe anemia with a hemoglobin level of 8.7 g/dL, a hematocrit of 20.7%, and a red blood cell count of 4.17 million/mm³. The total leukocyte count was within normal limits, although differential leukocyte count demonstrated neutrophilia. Red cell indices showed an MCV of 68.5 fL, and red blood cell indices were within the reference range except for a mildly elevated red cell distribution width (RDW) of 15%. Platelet count and coagulation parameters were normal. Biochemical investigations, including serum iron profile, vitamin B12 levels, and red cell folate concentrations, were within normal limits. Screening tests for autoimmune hemolysis, glucose-6-phosphate dehydrogenase (G6PD) deficiency, and malaria infection were negative, as evidenced by negative Coombs' test, G6PD assay, and malarial antigen test, respectively. Liver function evaluation demonstrated a mild elevation in total and indirect bilirubin levels, suggestive of ongoing hemolysis. Other biochemical parameters, including blood glucose, blood urea, serum creatinine, and electrolyte levels, were within normal limits. Ultrasonographic examination of the abdomen revealed cholelithiasis along with mild splenomegaly.

To further characterize the hemoglobin abnormality, high-performance liquid chromatography (HPLC) was performed. Hemoglobin analysis showed markedly elevated fetal hemoglobin (HbF) levels of 11.0% (reference value <1.5%), significantly reduced adult hemoglobin (HbA) levels of 5.1% (reference range 83.24–90.79%), and a markedly increased proportion of hemoglobin S (HbS) at 50.6%, which is absent in normal individuals. Based on these findings, a diagnosis of homozygous sickle cell disease (HbSS) was established. Subsequently, hemoglobin electrophoresis was recommended for the patient's immediate family members. Evaluation of both parents and the sibling revealed the presence of hemoglobin S in the heterozygous state (HbAS), consistent with sickle cell trait and supporting the hereditary nature of the disorder. Family screening identified both parents and one sibling as HbAS carriers. Among the three patients, this child exhibited the lowest HbF level and multiple chronic complications, suggesting a relatively severe disease phenotype.

Patient report

Bio-Rad	DATE: 05/17/2025
D-10	TIME: 10:20 PM
S/N: #DefInstIni	Software version: 4.30-2
Sample ID:	41752501952
Injection date	05/17/2025 09:49 PM
Injection #: 23	Method: HbA2/F
Rack #: ---	Rack position: 3



Peak table - ID: 41752501952

Peak	R.time	Height	Area	Area %
A1a	0.23	8177	30305	2.8
A1b	0.33	2248	8837	0.8
F	0.50	18138	116698	11.0
A1c	0.86	363	4869	6.1
P3	1.47	3387	19598	1.8
A0	1.69	5278	53424	5.0
A2	3.41	17903	299154	33.3 *
S-Window	4.17	124091	545217	50.6
Total Area:	1078101			

Concentration:	%
F	11.0
A1c	6.1
A2	33.3 *

Fig no1 hemoglobin electrophoresis graph report of case no1 showing elevated HBS peak

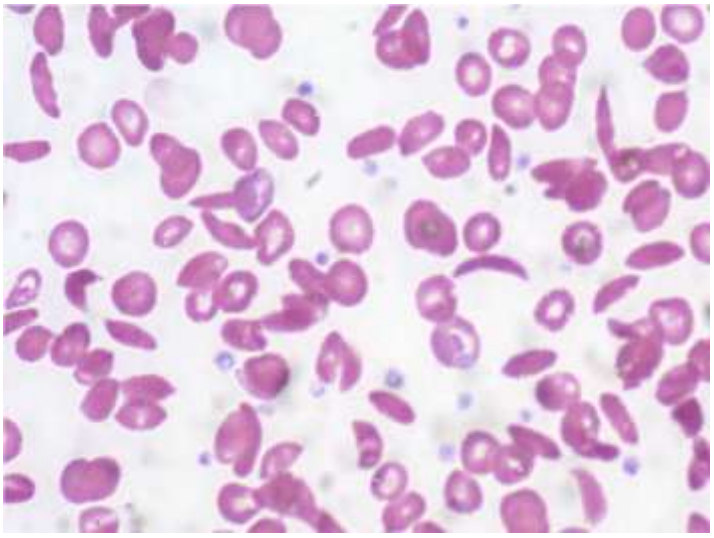


Fig no 2 blood smear shows sickle shaped RBC , target cells, microcytic RBC, elongated clls and acanthocytes in 100x

Case report no. 2

A 7-year-old girl presented to the Pediatric Outpatient Department with complaints of recurrent leg pain, abdominal pain associated with discomfort, and intermittent fever for the preceding three days. Her medical history was significant for hip surgery performed during early childhood. There was no history of any major comorbid illness.

On physical examination, the patient appeared pale, and mild splenomegaly was noted. Laboratory investigations revealed severe anemia with a hemoglobin concentration of 6 g/dL and a hematocrit of 22.7%. The total leukocyte count was within normal limits, although the differential leukocyte count demonstrated neutrophilic predominance. Red blood cell indices were largely within normal limits, except for a mildly elevated red cell distribution width (RDW) of 14.3%. Platelet count and coagulation parameters were normal. Further investigations showed a normal serum iron profile, vitamin B12 levels, and red cell folate concentrations. Coombs' test, glucose-6-phosphate dehydrogenase (G6PD) assay, and malarial antigen screening were negative. Liver function tests revealed a mild increase in total and indirect bilirubin levels, suggestive of ongoing hemolysis. Other biochemical parameters, including blood glucose, blood urea, serum creatinine, and electrolyte levels, were within normal limits.

Abdominal ultrasonography demonstrated mild splenomegaly without any other significant abnormality. To evaluate the underlying hemoglobinopathy, high-performance liquid chromatography (HPLC) was performed. Hemoglobin analysis showed markedly elevated fetal hemoglobin (HbF) levels of 36.7% (reference value <1.5%), markedly reduced adult hemoglobin (HbA) levels of 59.9% (reference range 83.24–90.79%), and a significantly increased proportion of hemoglobin S (HbS) at 36.5%, which is absent in healthy individuals.

Based on the clinical presentation and HPLC findings, a diagnosis of homozygous sickle cell disease (HbSS) was established. Subsequently, hemoglobin electrophoresis was recommended for the patient's family members. Evaluation of both parents demonstrated hemoglobin S in the heterozygous state (HbAS), confirming carrier status and supporting the inherited nature of the disease. The relatively high HbF concentration may have contributed to partial protection against severe vaso-occlusive manifestations, consistent with the recognized inhibitory effect of HbF on HbS polymerization.

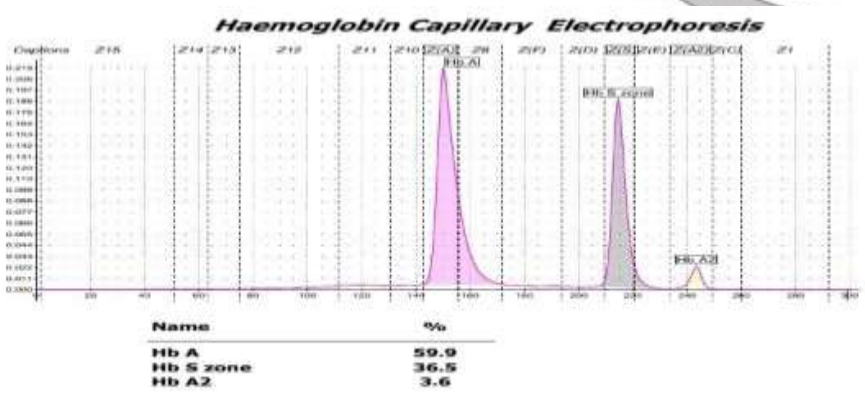


Fig no3 hemoglobin electrophoresis graph report of case no 2 showing elevated HBS peak and HBA

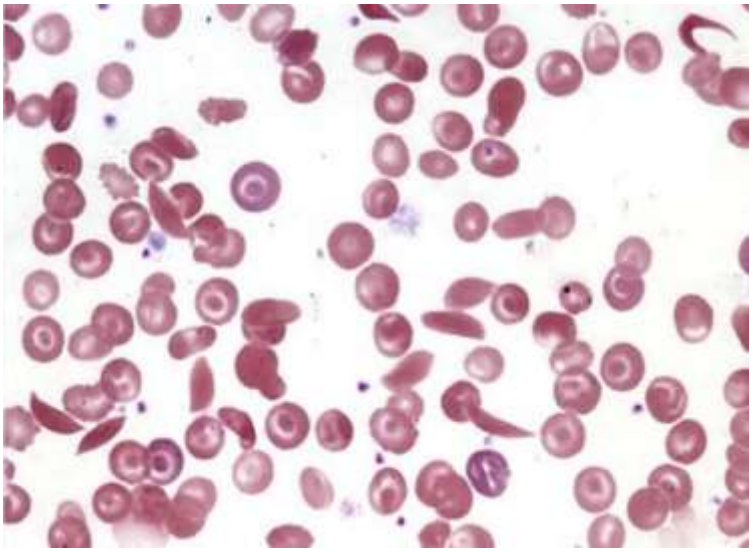


Fig no 4 blood smear shows sickle shaped RBC , target cells, microcytic RBC, spherocytes, elongated clls and acanthocytes in 100x

Case no. 3

A 10-year-old girl presented to the hospital with complaints of delayed wound healing for the past 15 days, accompanied by intermittent pain at the affected site. The symptoms developed following a fall from a bicycle approximately two weeks earlier. There was no history of jaundice or any other chronic medical illness. On physical examination, the patient appeared pale, and mild hepatomegaly was noted. Hematological investigations revealed anemia with a hemoglobin level of 8 g/dL and a hematocrit of 23.7%. The total leukocyte count was within normal limits. Red blood cell indices were largely unremarkable, except for an elevated red cell distribution width (RDW) of 17.8%. Platelet count and coagulation parameters were within the normal range.

Further laboratory evaluation demonstrated a normal serum iron profile, vitamin B12 levels, and red cell folate concentrations. Coombs' test, glucose-6-phosphate dehydrogenase (G6PD) assay, and malarial antigen testing were negative. Liver function tests showed mild elevations in total and indirect bilirubin levels, indicating

ongoing hemolysis. Renal function tests, including blood urea and serum creatinine, as well as blood glucose and serum electrolyte levels, were within normal limits.

Abdominal ultrasonography revealed mild hepatomegaly along with splenomegaly. In view of the clinical findings, hemoglobin analysis was performed using high-performance liquid chromatography (HPLC). The chromatographic profile demonstrated markedly elevated fetal hemoglobin (HbF) levels of 29.7% (reference value <1.5%), significantly reduced adult hemoglobin (HbA) levels of 5.4% (reference range 83.24–90.79%), and a substantially increased hemoglobin S (HbS) fraction of 72.9%, which is absent in healthy individuals. Based on the clinical presentation and HPLC findings, a diagnosis of homozygous sickle cell disease (HbSS) was established. Despite an elevated HbS fraction, the relatively high HbF level may have moderated the overall clinical severity, illustrating the heterogeneous phenotype observed in pediatric sickle cell disease. Hemoglobin electrophoresis was subsequently recommended for the patient's immediate family members. Screening of both parents and the sibling revealed hemoglobin S in the heterozygous state (HbAS), confirming carrier status and supporting the inherited nature of the disorder.

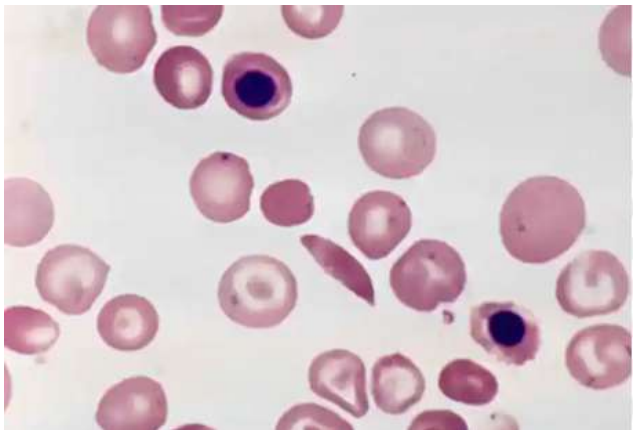


Fig no 5 blood smear shows sickle shaped RBC , target cells, microcytic RBC, spherocytes, elongated cells and n RBC in 100x

Table no 1: Comparative Summary Table Of All The 3 Case Reports

Parameter	Case 1	Case 2	Case 3
Age	8 years	7 years	10 years
Sex	Male	Female	Female
Hemoglobin	8.7	6	8
HbF (%)	11	36.7	29.7
HbS (%)	50.6	36.5	72.9
Splenomegaly	Yes	Yes	Yes
Hepatomegaly	No	No	Yes
Gallstones	Yes	No	No
Delayed wound healing	Yes	No	Yes
Hip surgery	Yes	Yes	No

Discussion:

Sickle cell disease (SCD) is an inherited hemoglobin disorder resulting from the homozygous inheritance of the sickle β-globin gene (βS). The disease arises due to a single nucleotide substitution in the β-globin gene, causing the replacement of glutamic acid by valine at the sixth position of the β-globin chain. This molecular alteration leads to the formation of hemoglobin S (HbS), which polymerizes under deoxygenated conditions, resulting in erythrocyte sickling, chronic hemolysis, and vaso-occlusive complications [5].

The present case series demonstrates the marked clinical heterogeneity of pediatric homozygous sickle cell

disease despite a shared molecular diagnosis. Although all three children were confirmed to have HbSS by HPLC, their clinical manifestations ranged from chronic leg ulceration and pigment gallstones to recurrent painful crises and delayed wound healing. These findings emphasize that disease severity cannot be predicted solely by the presence of HbS and is influenced by additional genetic and biological modifiers

SCD is widely distributed across sub-Saharan Africa, the Middle East, India, and parts of the Mediterranean region [6]. In India, the prevalence of the sickle gene is particularly high among tribal populations, scheduled castes, and other socioeconomically disadvantaged communities, with carrier frequencies ranging from 5% to 35% in certain regions[7,8.]

Historically, the sickle mutation has been associated with five major βS-globin haplotypes, namely Senegal, Benin, Bantu/Central African Republic, Cameroon, and Arab-Indian. These haplotypes offer useful information about the evolutionary history and geographic spread of the disease6. Variations among haplotypes are associated with differences in fetal hemoglobin (HbF) expression, with the Senegal and Arab-Indian haplotypes generally exhibiting higher HbF levels and consequently a milder clinical course [9.] More recent genomic studies, however, suggest that the sickle mutation may have originated from a single ancestral event rather than multiple independent occurrences.[10]

The clinical manifestations of SCD are highly variable and are influenced by both genetic and environmental factors. One of the most important disease modifiers is the concentration of fetal hemoglobin [11]. HbF inhibits the polymerization of HbS and reduces red cell sickling, thereby delaying disease manifestations during infancy. Consequently, individuals with persistently elevated HbF levels often experience a less severe disease phenotype . Conversely, a rapid decline in HbF may result in the appearance of symptoms at an earlier age than typically expected.[12]

One of the most notable observations in this series was the variation in fetal hemoglobin (HbF) levels among the three patients. HbF is a well-recognized inhibitor of HbS polymerization because it interferes with the intracellular aggregation of deoxygenated sickle hemoglobin. Previous studies have consistently shown that higher HbF concentrations are associated with reduced vaso-occlusive episodes, fewer hospitalizations, and improved survival. In our series, the patient with the lowest HbF level (11%) exhibited chronic complications, including leg ulceration and gallstone disease, whereas the patients with higher HbF levels demonstrated comparatively fewer chronic complications despite persistent anemia. Although definitive statistical conclusions cannot be drawn from three cases, the observed trend supports the established role of HbF as an important modifier of disease phenotype.

Clinical features of SCD vary according to age. Infants commonly present with nonspecific manifestations such as dactylitis, splenic sequestration, irritability, fever, and abdominal discomfort. As children grow older, more characteristic complications become evident, including recurrent painful vaso-occlusive crises, acute chest syndrome, severe infections, priapism, stroke, and other neurological complications. [13] The absence of certain classical manifestations, such as dactylitis, does not exclude the diagnosis, as disease expression varies considerably among patients.[14,15]

Infectious complications remain a major cause of morbidity in children with SCD. Functional asplenia predisposes affected individuals to infections caused by encapsulated organisms, particularly Streptococcus pneumoniae[16]. Invasive pneumococcal disease may occur despite prior vaccination, emphasizing the importance of continued surveillance and prompt antimicrobial therapy. Early recognition and treatment of bacterial infections significantly improve clinical outcomes [17].

The HPLC profiles further highlighted the value of quantitative hemoglobin analysis beyond confirming the diagnosis. In addition to demonstrating elevated HbS fractions, HPLC provided accurate measurement of HbF and HbA, enabling a more comprehensive assessment of disease biology. This information has potential prognostic significance because HbF levels may help identify patients who are likely to benefit from early initiation or optimization of hydroxyurea therapy. Our findings therefore support the incorporation of HPLC as both a diagnostic and prognostic tool in pediatric sickle cell disease.

Another important observation was the delayed recognition of sickle cell disease despite recurrent symptoms and previous complications. Two children had undergone orthopedic procedures, and one had already developed pigment gallstones before the diagnosis of HbSS was established. These findings highlight the consequences of limited newborn screening and delayed referral in resource-constrained settings. Earlier diagnosis could have facilitated preventive interventions, family counseling, vaccination, and disease-modifying therapy before irreversible complications developed.

The relationship between SCD and malaria is complex. While individuals carrying the sickle cell trait (HbAS)

derive substantial protection against severe malaria, patients with homozygous sickle cell disease (HbSS) remain susceptible to severe infection and its complications.[18] The protective effect observed in heterozygous individuals is thought to result from impaired survival of malaria parasites within sickled erythrocytes and enhanced clearance of infected red cells by the reticuloendothelial system.[19]

Family screening identified HbAS carrier status in first-degree relatives of all three patients, reinforcing the autosomal recessive inheritance pattern and emphasizing the importance of cascade screening. In regions with a high prevalence of sickle cell disease, systematic family evaluation following diagnosis of an affected child represents a practical strategy for identifying at-risk couples and reducing future disease burden through genetic counseling.

Although limited by the small sample size, this case series contributes clinically relevant observations by demonstrating the relationship between HPLC-derived hemoglobin fractions and the heterogeneous presentation of pediatric HbSS. The findings underscore the importance of integrating quantitative hemoglobin analysis, careful clinical assessment, and family screening into routine evaluation of children presenting with chronic anemia or recurrent painful episodes in endemic regions.

Advances in comprehensive care have markedly improved survival and quality of life among patients with SCD. Current management strategies include regular clinical follow-up, folic acid supplementation, vaccination, infection prophylaxis, and malaria prevention in endemic regions. [20]Disease-modifying therapies such as hydroxyurea have been shown to increase HbF levels, reduce vaso-occlusive episodes, and decrease hospitalization rates. Newer agents, including crizanlizumab, have further expanded therapeutic options. Chronic transfusion therapy is recommended for selected high-risk patients, particularly those with recurrent vaso-occlusive crises, stroke, acute chest syndrome, or evidence of severe organ involvement[21,22].

Despite these advances, access to specialized care remains limited in many low- and middle-income countries. Delayed diagnosis, inadequate screening programs, restricted availability of disease-modifying therapies, and limited transfusion services continue to contribute to adverse outcomes. Strengthening comprehensive care programs and improving access to diagnostic and therapeutic facilities are essential to reducing disease-related morbidity and mortality among children with sickle cell disease.

Conclusion:

The implementation of newborn screening programs for sickle cell disease in endemic regions, particularly in developing countries, should be prioritized to facilitate early diagnosis and timely intervention. Identification of affected infants during the neonatal period enables appropriate disease monitoring, preventive measures, and comprehensive clinical management, thereby reducing disease-related morbidity and mortality. Early detection also allows healthcare providers to initiate proactive care strategies and provide genetic counseling to affected families. Furthermore, infants and young children presenting with severe anemia, even in the absence of a positive family history, should be evaluated for sickle cell disease to avoid delayed diagnosis and improve clinical outcomes.

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