REVIEW OF LITERATURE
The fundamental cause of sickle cell disease is the decreased deformability of the sickled red cell produced by gelation of hemoglobin S. Sickle cell disease (SCD) is clinically one of the most important haemoglobinopathies. It is characterised by haemolytic anaemia, an increased susceptibility to infections and vaso-occlusion that occurs in almost all vascular beds leading to ischaemic tissue injury with organ dysfunction and early death. Outcome is difficult to predict, and few effective therapeutics are available.

3.1 PREVALENCE OF SICKLE CELL DISEASE : In India, haemoglobinopathies, especially sickle haemoglobin are the commonest genetic disorders in the tribal belt of Central and Southern India. As reported by R. B. Gupta\textsuperscript{44} in undivided Madhya Pradesh (Madhya Pradesh and Chhattisgarh) harbours the largest tribal population in India, which is about one fourth of the total tribal population of the country. These tribal groups are characterized by their unique socio-cultural and religious practices and follow strict endogamous practice. These tribal populations are stated to be aboriginal population of India. Sickle haemoglobin was first discovered from a tribal population of Nilgiri Hills of South India in. Later, it was reported from the tribal population of Central India i.e. Madhya Pradesh and Chhattisgarh and its surrounding areas falling in the states of Rajasthan, Gujarat, Maharashtra, Andhra Pradesh and Orissa. This led to an impression/belief that sickle haemoglobin is confined to tribal populations/ belt. Later, in some tribal groups like Bhils of Jhabua district, tribal groups of Bastar and Pradhans of district Mandla, Halbas of districts of Rajnandgoan and Durg, the prevalence rate of sickle haemoglobin have been very high i.e. over 30 percent. The prevalence of sickle haemoglobin from various parts of Madhya Pradesh and Chhattisgarh varied from 15 to 30 percent. It was found that the non-tribal people of HbS belt especially Scheduled Castes and other Backward class communities have sickle haemoglobin in similar proportion as that of tribals of the area. We have also found prevalence of b-thalassaemia in various proportions, average 2-4 percent, among the various tribal populations. In some pockets, Scheduled Castes populations e.g. Jharia, Mehra,
Dahariya etc. have very high prevalence of sickle haemoglobin i.e. over 30 percent. Co-inheritance of b-thalassaemia gene along with gene for sickle haemoglobin also causes sickle cell disease. There are large variations in prevalence of sickle haemoglobin in various tribal/Scheduled Caste populations of a geographical area/district and within a tribe/ caste population scattered over a large area. Gond and Bhil group of tribal constitute large proportion of tribal population of the state. Among Gond group of tribal the prevalence rate of sickle haemoglobin generally varies from 10 to 25 percent whereas in the Bhil group of tribal the prevalence rate varies from 15 to 33 percent. Earlier studies carried out by various workers show that in Madhya Pradesh the Scheduled Caste and Backward Class communities of the tribal predominant area also have sickle cell gene in almost similar proportion (Unpublished reports). As this disorder is recessive in nature the heterozygotes are absolutely asymptomatic but the homozygous suffer from serious complications leading short life span. The transmission of the disorder depends on the marriage patterns / customs of the population or the affected person.

Fig No. 3.1. (Out of 45 districts of the state, 27 districts fall under the sickle cell gene belt. These districts arranged in descending order according to percentage of ST and SC population) are Jhabua, Barwani, Dindori, Mandla, Dhar, Shahdol, Umaria, Betul, Seoni,West Nimar, Chhindwara, Harda, East Nimar, Jabalpur, Ratlam, Dewas, Katni, Damoh, Hoshangabad, Sagar, Satna, Balaghat, Ujjain, Indore, Mandsaur, Neemuch and Narsimhpur.)
Patients with SCD can develop specific and sometimes life-threatening complications, as well as extensive organ damage reducing both their quality of life and their life expectancy. Proven effective treatment options for sickle cell patients are limited to hydroxyurea, blood transfusions and bone marrow transplantation. With the increasing prevalence of SCD in Madhya Pradesh, a fundamental understanding of its pathophysiology and clinical syndromes is very important.

3.2 PATHOGENESIS OF VASOOCCLUSION:

Episodic vascular occlusion is the hallmark feature of sickle cell disease. From the perspective of physiology, this event interrupts or retards blood flow, causing deprivation of tissue oxygen delivery. From the perspective of clinical observation,
vasoocclusive events such as the acute painful episode occur sporadically and with remarkable variability of frequency, regularity, and severity in and among individuals. Beneath the threshold of clinical detectability, however, are subclinical vasoocclusive events involving multiple organs, as discussed in separate chapters elsewhere in this volume. The frequency and importance to chronic organ damage of the undetected subclinical occlusive episodes are unknown. Similarly undefined is the significance of chronically impaired blood flow.

The uncertainties regarding vasoocclusion extend to its pathogenesis. It is agreed that the sine qua non of sickle cell vasoocclusion is the mutant gene product Hb S with its resultant pathological effects on sickle erythrocytes. However, attributing vasoocclusion entirely to Hb S polymerization and sickling of sickle red cells is an article of faith derived from the original hypothesis of Ham and Castles\textsuperscript{45}, which was based on logical synthesis rather than direct evidence. Even today, much of what is “known” about vasoocclusion in sickle cell disease is based on inference and extrapolation form the pathological properties of Hb S and sickle erythrocytes in vitro.

The central eminence ascribed deoxyhemoglobin S insolubility and cellular sickling has resulted in presumed mechanisms for acute painful episodes, the relationship of which to events in vivo has not been documented clearly. There is no direct evidence that individuals experiencing a typical acute painful episode have undergone any of the physiological changes known to influence polymerization or sickling in vitro. The same caution required not to attribute singular causality to more recently discovered physiological perturbation, such as activated coagulation processes, enhanced sickle cell-endothelial cell adherence, disturbed vascular reactivity, or exaggerated sickle red cell dehydration. Changes in each of this variables has been reported to accompany acute pain in sickle cell disease, but the proximate cause of vascular occlusion remains to be determined. Although the “vicious cycle of erythrostasis” remains the accepted explanation for painful vasoocclusion\textsuperscript{46}, the mechanisms participating in this process are more numerous, complex, and interdependent than originally believed. A more thorough comprehension of the varied mechanisms that probably contribute to vasoocclusion in sickle cell disease may lay the foundation for improved therapy.
Neither experimental nor clinical data have provided unambiguous proof of this concept. Two observations provide the strongest evidence for polymerization-induced red cell sickling as the preeminent pathophysiological process in vasoocclusion. The first is that compound heterozygosity for hemoglobins S and C results in sickle cell disease rather than sickle cell trait, despite Hb C resembling Hb A in ability to impede polymer information. This difference in clinical severity apparently is related to the greater fractional content of Hb S and the marked dehydration of Hb SC red cells compared with sickle cell trait red cells, implying that the clinical disease is the result of concentration-enhanced polymerization. The second example is provided by the ameliorated clinical severity observed in Hb S-hereditary persistence of fetal hemoglobin, in which the pan cellular high level of Hb F inhibits polymerization and cellular sickling. Thus, the greater clinical severity of syndromes having higher intraerythrocytic concentrations of Hb S provides the main clinical evidence that polymerization is an important determinant of vasoocclusion severity.

Other evidence for the importance of sickling-induced vasoocclusion is less persuasive. Based on the observation that a calculated estimate of polymerization tendency for the “average cell” correlates with both the global level of anemia and an undocumented supposition of clinical severity among different sickle cell genotypes, it has been argued that Hb S polymerization is the major effector of pathogenesis. The variability of clinical severity within genotypes is at least as great as variation between genotypes, so the meaning of such comparisons is open to question. Clinical improvement during hydroxyurea therapy has been attributed to elevated levels of Hb F inhibiting polymerization. However, hydroxyurea also reduces the proportions of poorly deformable dense red blood cells, highly adhesive erythrocytes, and white blood cells, any of which might affect clinical improvement independent of sickling tendency. Moreover, the improved level of anemia induced by hydroxyurea has been observed prior to levels of Hb F increasing, demonstrating that the retardant effect of Hb F on polymerization is not essential to the therapeutic effects of hydroxyurea. Indeed, evidence that sickling is unlikely to be the sole determinant of acute vasoocclusion derives from the lack of correlation between painful event and the number of dense cells, which would support the greatest degree of hemoglobin
polymerization, and from the only weak correlation between painful episodes and Hb F levels\textsuperscript{52}. This assessment of the importance of Hb F level is confounded by the heterocellular distribution of Hb F and the single-file passage of cell through the smallest vessels. Thus, on a cell-by-cell basis, likelihood of vasoocclusion being initiated is complex, highly stochastic process the vagaries of which have been enunciated clearly.

There is clinical evidence that sickling appears to be the primary and fully sufficient precipitant of vasoocclusion in specific situations, such as in hypertonic/hypoxic renal environment, low ambient oxygen tension, and the use of hypertonic radiographic contrast material. However, in many other circumstances vasoocclusion may derive from other antecedent events such as hemostatic activation, poor red blood cell deformability, endothelial adhesivity, and increased vascular tone, with sickling occurring as secondary event. Our view is that genetically determined tendency for polymerization provides first approximation of clinical severity so that condition in which Hb S is diluted by high level of Hb F, Hb A, Hb C, or other non polymerizing hemoglobin species are generally milder than those in which Hb S is not diluted, but that additional modulating factors dictate moment-to-moment and patient-to-patient variability\textsuperscript{53}. Thus, cellular sickling can be sufficient to achieve vasoocclusion, but it is uncertain whether sickling is both necessary and sufficient for and the primary initiator of ordinary acute vascular occlusion.

Sickle cell disease is assumed to entail microvascular obstruction, since the smallest vessels should be most susceptible to plugging regardless of actual mechanism. Surprisingly, however, there are few data regarding the actual location of vasoocclusion.

Autopsy and biopsy findings are not reliable indications of the site of vasoocclusion, as obstructed vessels may represent post-mortem or fixation artefacts. Observations of in vivo nail bed flow in patients with sickle cell disease revealed interruption of flow at bifurcations and actual capillary occlusion\textsuperscript{54}, perturbations that were transient and not significantly greater than controls intermittency of microvascular flow in the forearm skin of subjects with sickle cell disease was observed using laser-Doppler assessment of flow\textsuperscript{55}, but experiments using the same
methodology\textsuperscript{56} determined that intermittent flow was not found uniformly over the forearms of subjects with sickle cell disease and was found in normal subjects as well. Totally occluded capillaries were observed in the rat mesocecum perfused with sickle red cells\textsuperscript{57}.

Vascular occlusion may be initiated in other vascular sites. In the perfused rat mesocecum, most red cell adherence occurred at the level of postcapillary venules\textsuperscript{57}, suggesting that vasoocclusion is initiated at this site and mediated by endothelial cell adherence. In contrast, it has been argued that vascular obstruction should occur at the precapillary arteriole as a result of direct plugging by polymer-containing, less deformable sickle red cells. Cell trapping at locations such as arteriolar-capillary bifurcations and venular junctions has been observed in microvascular flow models\textsuperscript{58, 59}. Emphasizing the effects of hemostatic activation and endothelial perturbation, some have argued that vascular obstruction may involve large vessels, at least in some organs such as the central nervous system and perhaps lung\textsuperscript{60}. Large vessel obstruction has been well documented by cerebral arteriography in sickle cell subjects after strokes.

Clearly, there is inadequate documentation of the locus of initiation of the vasoocclusive event.

\textbf{3.2.1 STEADY STATE & ACUTE EPISODE} : Clinical observation has provided the concept of a “steady state” in which individuals with sickle cell disease are well until something, usually assumed to be “sickling,” precipitates an acute vasoocclusive event. However, as biochemical data become more detailed, the distinction between a steady state and an acute event is becoming blurred.

For example, a number of biochemical abnormalities develop variably, sporadically, or chronically in the blood of sickle patients who are remote from acute painful episodes. Of particular interest, C-reactive protein, a stimulant of tissue factor expression by monocytes\textsuperscript{63, 64}, is occasionally elevated, and its level during the steady state is reported to fluctuate more in those sickle patients who have more frequent acute painful episodes\textsuperscript{62}. Levels of fibrinogen and other acute phase reactants\textsuperscript{61, 62}, tumor necrosis factor\textsuperscript{65}, interleukin-1\textsuperscript{65}, and endotoxin\textsuperscript{66} are each elevated occasionally
in the plasma of sickle cell disease patients regarding the roles and levels of cytokines and other biological modifiers of endothelium, macrophages and other old cells in the generation of vasoocclusion.

It has been assumed that the fluctuations of acute phase reactants during steady state conditions reflect subclinical vasoocclusive events, but it is not known whether these are simply biochemical responses of no pathogenic importance. A related assumption is that the level of any factor relevant of pathophysiology must rise in immediate juxtaposition to the acute vasoocclusive episode. There, of course, is not a priori reason for this. Given the current lack of accurate information as to details of initiation and progression of vasoocclusion, this argument is neither scientific nor rigorous.

There may be relatively little difference in the physiological conditions of the "steady state" and the acute episode. Development of an acute painful episode could simply reflect a slight tipping of the balance of circulatory homeostasis, derived simply from a stochastic accrual of input from multiple potentially relevant physiological or pathological factors. The phrase stochastic stasis may be apt.

3.2.2 OTHER POSSIBLE CAUSE OF PATHOPHYSIOLOGY OF SICKLE VASOOCCCLUSION: At present, insufficient data are available to justify ascribing vasoocclusion to a single or simple process. Indeed, it seems fatuous to do so, given the remarkable variety of potentially relevant abnormalities indentified. Moreover, it is entirely possible that the mechanism of vasoocclusion varies over time and anatomical site and among patients. Certainly, there are relevant differences among distinct vascular beds, as shown by the functional heterogeneity of endothelial cells from large-vessel and microvessel sources and by organ-to-organ differences in microvascular architecture. Organ-specific variance in mechanisms of vasoocclusion also provides a possible explanation for the reportedly greater prevalence of retinopathy and bone infarcts in Hb SC disease and sickle cell anemia with coexistent αthalassemia. It is further conceivable that the pathogenic mechanism varies from one complication to another, so that clinical, biochemical, and correlative data obtained from patients having acute painful episodes may not reflect...
the same pathophysiology as vasoocclusive processes in specific organs. These possibilities have not been addressed by adequate experimentation or clinical observation. May be there are multiple pathophysiologies has two distinct implications. First, the possibility exists that vasoocclusion always is multifactorial, involving various processes. Second, different mechanisms are likely to prevail as primary precipitants under different circumstances. During inflammation, increased leukocyte interactions with-and influences on-endothelium could as the triggering event, impede microcirculatory flow, promote deoxygenation and acidosis of sickle cells, enhance polymer formation, and promote sickling. Under other circumstances, stimulation of tissue factor expression on macrophages may result in thrombin generation and hemostatic initiation of vasoocclusion. In response to platelet activation or von Willebrand factor, respectively obstruction by triggering red cell adherence to endothelium. Other valid mechanistic schemes are supported by published data.

![Diagram](image)

**Fig. No. 3.3 Proximate factors relevant to hemoglobin polymerization as a determinant of vasoocclusion.**

### 3.2.3 RISK FACTORS FOR DEVELOPMENT OF SICKLE VASOOCCCLUSION

#### 3.2.3.1 Hemoglobin Polymerization

The manner in which Hb S polymerization exerts its effect on vasoocclusion has been widely misconstrued. Many believe that episodic complications of sickle cell disease are the result of fierce gusts of cellular...
deoxygenation and sickling. However, every red blood cell ventures from the arterial circulation and is deoxygenated to some degree at least four times a minute. With this frequency sickle red cells acquire deoxy Hb S, the extreme insolubility of which results in the generation intraerythrocytic polymer. There is experimental evidence that the generation of even small amounts of Hb S polymer is associated with detectable cellular deformation \(^{71}\) and that substantial numbers of sickle red cells become grossly sickled during passage from the arterial to the venous blood. Therefore, “sickling” is a chronic ongoing process, each cell sickling to some extent an average of 6,000 times a day. Clearly the relationship between an event of this frequency and the profoundly less frequent painful vascular occlusions is more complex than simple cause and effect. Moreover, according to the kinetic theory of polymerization there is a delay between the generation of deoxy Hb S and initial gelation \(^{46}\), so that many sickle red cells fail to sickle until they have passed into a vessel of the venous system too large to be occluded by sickled cells. Certainly usual painful episodes are not the result of patients experiencing periods of hypoxia, acidosis, or hyperosmolarity. Finally, regarding the two presumed clinical consequences of sickling, it is apparent that vasoocclusion involves more complex mechanisms that red cell sickling alone and that exacerbations of anemia are seldom related to accelerated rates of sickling and hemolysis.

Nevertheless, the polymerization that causes erythrocyte sickling has been exhaustively studied in vitro and has been described by two theories. One argues that the gelation of Hb S occurs so rapidly that polymer formation is dominated by thermodynamics and the equilibrium polymer concentration. The other argues that the system is never in equilibrium and is dominated by the kinetics of polymerization and oxygen transits \(^{46}\). Ignoring kinetic constraints on polymerization is valid only in sickle cells in which equilibrium conditions are created in vitro and in sickle cells in which polymer persists as deoxygenation begins in vivo. Because oxygenation-induced depolymerization occurs without delay, except in a minority of severely dehydrated cells with extremely high mean corpuscular hemoglobin concentration, oxygenated sickle cells contain no polymer when deoxygenation begins. Thus, equilibrium
considerations pertain to a dehydrated, dense minority of cells, but kinetic considerations best characterize polymerization in the majority of sickle cells.

Understanding either version of polymerization requires considerations of red cell membrane factors. The equilibrium theory pertains to polymerization in the most dense cells that are most likely to generate polymer by virtue of their extreme hemoglobin concentration; it is thus dependent on the pathways that lead to cellular dehydration. The sensitive balance between very short microlonger delay times to polymerization, which is critical to the kinetic understanding of polymerization in most sickle red cells, can be perturbed by cellular factors that delay transit time, such as endothelial adhesivity or poor cellular deformability.

The possible direct influence of sickle red cell membrane on intraerythrocytic sickle polymerization remains unsettled. The promotion of anisotropic polymer formation by open, but not resealed, sickle cell ghosts suggests a membrane effect, which may explain why sickle erythrocytes seem to contain preexisting nucleation sites. On the other hand, careful analyses of polymerization within individual cells determined that the kinetics were identical to those in solutions having the same hemoglobin concentrations and compositions, suggesting influence. Moreover, studies of the effect on polymerization of inside-out membrane vesicles from normal red cells, used to circumvent the unavoidable artifacts associated with preparing sickle red cell ghosts, demonstrated that membranes had no material effect on kinetics. However, the possible roles of the abnormal hemoglobin deposits and clustered sites for hemoglobin binding on sickle red cell membranes in influencing polymerization suggest that this matter has not been completely resolved.

3.2.3.2 Blood Viscosity and Rheology: The importance of viscosity and rheology to the flow of sickle blood is evident from a variety of experiments in vitro and from clinical experiences. Increasing the hematocrit of sickle cell blood in vitro, even with nonsickled red cells, increases its viscosity and impairs its flow properties. Such data provide the basis for the clinical recommendation of avoiding hematocrit levels greater than 30 percent when transfusing patients.

Clinical experiences also serve to demonstrate the importance of blood viscosity on vasoocclusive events. The occurrence of painful vasoocclusion has been
observed after the cessation of oxygen inhalation coincided with increasing hematocrit levels, as well as with numbers of white blood cells, irreversibly sickled cells, and reticulocytes and at at time when hematocrit levels by environmental exposure to carbon monoxide. The importance of blood viscosity to flow properties of whole blood is well illustrated by the disparate interaction of α thalassemia with sickle cell anemia, wherein salutary effects of α thalassemia on deformability of individual sickle red cells ameliorate the severity of anemia, but resultant higher hematocrit levels impair flow properties of sickle cell blood. The clinical consequences are more frequent retinopathy, osteonecrosis, and painful vasoocclusion. Thus, it appears that the potential benefits to blood flow of improved cell deformability are associated with changes in bulk viscosity that are to the overall detriment of blood flow. A further example of this apparent discrepancy is the positive correlation between better cellular deformability and frequency of painful episodes.

Fig 3.4 Determinants of deficient red cell deformability as a risk factor for vasoocclusion.

3.2.3.3 Dense Cells: Because of their higher intra-erythocytic Hb S concentration, the most-dense cells are least deformable, most likely to accrue polymer, and predicted to have the greatest difficulty negotiating the microcirculation, a prediction for which there is experimental evidence. Obstruction by the dense cell fraction develops occasionally at arteriolar-capillary junctions in model systems. On the other hand, it is notable that frequency of vasoocclusive manifestations correlates not
with poor red cell deformability but with better deformability direct gate-keeping role for dense cells. It follows that the role of dense cells may be to logjam behind an obstruction rather than to initiate the vascular plug. Indeed, propagate, but do not initiate, vasoocclusion\textsuperscript{57,59}. The proportion of dense cells increase immediately before an acute crisis\textsuperscript{61,81} and falls during the first days of pin\textsuperscript{61,81}. It is not clear whether the due to an initial accumulation of dense cells or to a depletion in less-dense cells; similarly, the subsequent decreased fraction of dense cells could reflect either depletion of more-dense cells or increased production of less-dense cells.

3.2.3.4: Hemostatic System The most notable aspects of sickle cell coagulopathy are increased thrombin generation and platelet activation. These events generate factors that may potentiate vasoocclusion, including fresh thrombus, lipid surfaces that accelerate coagulation, cytoadhesive proteins that mediate red cell adherence (e.g., thrombospondin), and factors that modulate endothelial cell function and surface character (e.g., transforming growth factor \( \beta \) and platelet-derived growth factor). The true role of hemostatic changes is obscured by the considerable uncertainty regarding whether observed changes are causal versus secondary in nature. Regrettably, data regarding the role of the hemostatic system in vasoocclusion are descriptive. An additional hypothesis requiring investigation is the possibility that endothelial dysfunction, perhaps from repeated molestation of the endothelium by biological modifiers and adherent sickle red cells, underlies some part of this hemostatic aberrance. The speculation that stimulation of tissue factor expression on circulating monocytes could be the primary trigger of hemostatic activation in the patient with sickle cell disease is elaborated.
3.2.3.5. Red Cell Adhesivity: The propensity for sickle red cells to adhere to vascular endothelial cells has been documented in a variety of in vitro systems. The tenacity of this adhesion, the location and circumstances of its development, and the nature of the various adhesion mechanisms argue that this process could be an important participant in sickle vasoocclusion. Initiation of vasoocclusion by adherent cells, as suggested in the perfused rat mesentery model, may explain the fractional reduction in least-dense, most adherent red cells during the initial stages of painful vasoocclusion. This is consistent with the frequency of painful events correlating with better red cell deformability rather than vice versa. The cells in the least-dense fraction, capable of exhibiting receptor-mediated interactions with plasma proteins and endothelial cells, are thus attractive candidates as initiators of vasoocclusion. Recent data further suggest that patients with sickle cell disease are more susceptible to adherence-mediated vasoocclusion as a result of their red cells containing a greater proportion of receptor bearing stress reticulocytes. While reticulocytes appear to be the most adherent subpopulation of sickle cells, all subpopulations possess some levels of increased adhesivity.
3.2.3.6 **Endothelial Factors**: The endothelial cell itself may contribute to sickle vasoocclusion. Insofar as erythrocyte adherence plays a role in vasoocclusion, the active participation of the endothelial cell surface must be acknowledged. The vascular lumen is lined with a multitude of potentially relevant molecules, such as cyto-adherence receptors and factors having critical hemostatic roles. Acting as endothelial cell agonists, biological modifiers can alter receptor expression, increasing adherence of sickle red cells in-vitro and altering the delicate procoagulant-anticoagulant balance of the endothelial cell surface. It is entirely conceivable that alterations in the endothelial surface in response to concurrent illness augment the risk of vasoocclusion. Intriguingly, examination of retinal vessels from sickle patients suggests increased expression of intercellular adhesion molecule-a\(^8\)
, suggesting prior stimulation by agents such as tumor necrosis factor or interleukin -1 and concomitant expression of vascular cell adhesion molecule. Endothelial cells infected with herpes simplex virus in vitro\(^8\)
 suggests the possibility that clinical viral infection could activate endothelial cells to potentiate vasoocclusion.

3.2.3.7. **Vascular Factors**: Alterations in microvascular dynamics, blood flow, or vascular tone probably play a role in sickle vasoocclusion by affecting the distribution and rate of blood flow. Such events could either fulfill a critical primary role or
become important during evolution of vascular occlusion initiated by some other factor. Relevant observations from patients with sickle cell disease include oscillatory microvascular flow and similar flow intermittence during hyperemia. The potential role of specific vasodilatory and vasoconstricting agents remains unexplored, except to note that endothelin levels are preliminarily reported to be elevated in sickle cell disease and the mechanisms responsible for regulating its production are under investigation. The existence of different possible vascular sites for vasoocclusion raises the potential for immense heterogeneity of vascular regulation and endothelial cell responses in this process.

3.2.3.8. White Blood Cells: A contributory role for white blood cells in sickle cell vasoocclusion cannot be excluded and has received little investigative attention. Theoretically, several important influences of white blood cells can be envisioned. For example, the slow passage of the granulocyte, which is much less deformable than is the normal erythrocyte, through small vessels would be expected to slow microvascular red cell flow. Thus, enhancement of leukocyte-endothelial cell interaction due to infection, for example, might be sufficiently obstructive to allow sickling or adhesion of red cells in areas proximal to retarded flow. Despite this merely speculative understanding of the mechanisms by which leukocytes may influence vasoocclusion, a higher mortality rate in patients with sickle cell disease who have higher white blood cell counts has been observed. Interestingly, the beneficial effects observed with hydroxyurea therapy of sickle cell disease coincide with reduced white cell counts. Also provocative in this regard, painful vasoocclusion occurring after the cessation of oxygen inhalation coincided with increased numbers of white blood cells, as well as irreversibly sickled cells and reticulocytes, in the blood. We predict that the multiplicity of leukocyte influences on blood flow will be proven to exert major influences on sickle disease pathophysiology.

3.2.3.9. Erythrocyte Factors: Various cellular factors have some degree of influence. Most clearly, the concentration-dependent reduced oxygen affinity and elevated 2,3-bisphosphoglycerate levels within sickle red cells promote Hb S deoxygenation. This
adaptation, which normally enhances tissue oxygen delivery, may have the opposite effect in sickle cell disease by virtue of enhancing Hb S deoxygenation and polymerization, cellular sickling, and vascular occlusion.

A little-appreciated influence on the vasoocclusive process pertains to a hypothetical negative feedback loop involving polymerization and hemolysis. Heme catabolism is the only metabolic process of human cells that generates carbon monoxide, and hemolysis results in increased production of carbon monoxide. Elevated levels of carbon monoxide-Hb S in the blood of nonsmoking sickle cell patients represents a liganded form of Hb S that is stabilized in the relaxed quaternary conformation. The carbon monoxide ligand induces increased hemoglobin-oxygen affinity, decreased oxygen-carrying capacity, and greater solubility of Hb S. Thus, the carbon monoxide produced by hemolysis may modulate sickle cell pathophysiology.

2.2.3.10 Environmental Factors: Plasma factors also can influence intracellular conditions of the red cell. Since the erythrocyte is a perfect osmometer, the hyperosmolar conditions experienced during some types of clinical dehydration can raise intracellular Hb S concentration and thereby promote sickling. Systemic acidosis is also a hazardous clinical condition, because low pH levels reduce Hb S solubility and dehydrate sickle red cells. Insofar as erythrocyte adhensivity is mediated by plasma proteins, fluctuating levels of those that respond as acute phase reactants during concurrent illness portend adverse results. The plasma environment probably is responsible for the deficient red cell levels of zinc, ascorbate, and vitamin E characteristic of patients with sickle cell disease. The influence of cytokines and biological modifiers on endothelial function has been discussed elsewhere in this volume. Regional differences in pH and pO₂ may also influence vascular occlusion. Both are low in the spleen and renal medulla, and the latter also provides a hyperosmolar environment. Even sickle trait patients have impaired urine concentrating ability and occasional splenic infarcts. It is perhaps remarkable that patients with sickle cell disease preserve splenic function as long as they do and maintain any degree of renal function.
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2.2.3.11. Psychosocial Factors: Perception of pain, coping skills, personality, mood, and relationships with the health care system inevitably contribute to the pain of acute vasoocclusive events. The intimate interdependence of "psychological" and "physiological" aspects of pain render their contributions indistinguishable. One example of their coordinate participation relates to pain or emotion-stimulated release of vasoactive substances that mediate and modulate painful stimuli. Correspondingly, successful intervention with patients having inadequate coping strategies serves to diminish the frequency of painful experiences and their attendant morbidity.

3.3 PATHOGENESIS OF HEMOLYTIC ANEMIA: Increased red cell destruction and the attendant chronic, life-long anemia are important clinical manifestations of sickle cell disease. The absence of anemia at birth and its onset paralleling the postnatal fall in Hb F and concurrent emergence of $\beta^S$-globin synthesis implies that sickling of red cells is requisite for inducing the cellular abnormalities responsible for red cell destruction. The remarkable variation in the degree of anemia of different genotypes, within the same genotype, and even in the same individual over time suggests that multiple mechanisms are involved in mediating red cell removal from circulation. In vitro studies, indeed, have identified a number of plausible mechanisms that may be responsible for premature sickle red cell destruction. However, in spite of the wealth on in vitro data currently available, it is not possible to determine definitively the relative contribution and importance of the various mechanisms for increased red cell destruction in vivo.

Decreased hemoglobin and hematocrit values, increased reticulocyte count, and decreased red cell life span—all features of hemolytic anemia—are present in sickle cell disease. Intramedullary destruction of erythroid cells, which contributes significantly to the reduced hemoglobin levels seen in $\beta$ thalassemia, does not appear to be a significant contributor to the hemolytic process in sickle cell anemia. Both extravascular and intravascular hemolysis appear to contribute to this clinical features of the disease. Two mechanisms appear to contribute to sickle red cell destruction via extravascular hemolysis: monocyte and macrophage recognition and phagocytosis of sickle red cells that have undergone sickling- or oxidation-induced membrane
changes and physical entrapment of rheologically compromised red cells. Proposed mechanisms responsible for intravascular hemolysis include lysis of complement-sensitive red cells and hemoglobin loss during sickling-or shear-induced membrane fragmentation. It has been estimated that one-third of the total hemolysis occurring in sickle cell anemia is intravascular.

A strong correlation between percentage of irreversibly sickled cells—a morphologically distinct subpopulation of dense, dehydrated cells—in the circulation and reduction of red cell life span has been noted. Further studies have verified this observation, and in general the percentage of dense, irreversibly sickled cells does correlate with the degree of anemia. In support of this conclusion is the finding that in sickle cell disease genotypes with reduced numbers of sense, irreversibly sickled cells such as Hb S-β thalassemia and sickle cell anemia with coexistent α thalassemia the degree of anemia is less pronounced. Also, the increased hemoglobin level induced by hydroxyurea therapy is associated not only with higher Hb F levels, but also with decreased numbers of dense, irreversibly sickled cells. The findings that the dense sickle cell population has increased levels of surface-bound immunoglobulin, which leads to their recognition by monocytes and macrophages, and that these cells are also more sensitive to complement lysis suggest that this subpopulation of red cells is a key contributor to the hemolytic component of the sickle cell disease.

An issue that has not received sufficient attention in this regard is the relationship between the cell dehydration that leads to increased cell density and the membrane structural changes that lead to generation of morphological changes characteristic of irreversibly sickled cells. While it is true that a large fraction of irreversibly sickled cells are dehydrated with cell hemoglobin concentrations greater than 38 g/dL some of these cells are not as extensively dehydrated. Irreversibly sickled cells with a normal state of hydration and cell hemoglobin concentration in the range of 30 to 38 g/dL are frequently found in blood samples from individuals with sickle cell disease. Furthermore, not all cells found in the high-density fractions of sickled cells. A varying portion of these high-density cells exhibit discoid morphology (up to 30 percent). Thus, it is important to distinguish the hemolytic contribution due to increased cell density per se from that due to membrane changes that may
accompany cell dehydration. The dominant contributors to increased red cell destruction in sickle cell disease are likely to be the proximate sickling-unsickling and oxidation-induced membrane changes that promote cell dehydration rather than cell dehydration itself. Evidence that cellular dehydration alone is insufficient to support rapid hemolysis is evident from the degree of hemolytic anemia in Hb SC and Hb CC diseases being much less pronounced than that in sickle cell anemia, despite the more pronounced cell dehydration Hb SC and Hb CC diseases\textsuperscript{103}. This comparison, although ignoring the contribution of Hb S gelation, leaves open the possibility that membrane changes may be more important than dehydration. This tentative conclusion is yet to be validated by documenting the diminished presence of the implicated membrane changes in Hb SC disease. Understanding the membrane changes responsible for red cell surface changes will contribute to a detailed understanding of the mechanisms responsible for premature destruction of sickle cells.

Very early descriptions of erythrophagocytosis by tissue macrophages in sickle cell disease\textsuperscript{104} led to investigation of cell membrane changes that are involved in sickle cell recognition by macrophages. A number of studies have documented increased binding of immunoglobulin to sickle red cells\textsuperscript{100,102} and have suggested that this membrane-bound immunoglobulin could be a critical recognition signal. An important feature of these observations is that there is a great deal of heterogeneity in the amount of immunoglobulin bound to sickle red cells in an individual patient as well as to red cells among different patients. In terms of individual subjects, the amount of membrane-bound immunoglobulin increases with increasing cell density, and erythrophagocytosis is greatest when macrophages are fed the most-dense sickle red cell subpopulation.

Sickling-unsickling-induced membrane alterations in conjunction with associated oxidative damage are the most likely causes for the surface changes responsible for increased immunoglobulin binding. Recent studies in vitro have indeed shown that either repeated cycles of Sickling-unsickling or induced oxidative damage can generate red cell membrane surface alterations that lead to increased immunoglobulin binding and recognition by monocytes and macrophages\textsuperscript{93,105}. These observations, coupled with the findings that sickle red cell phagocytosis in vitro can...
be inhibited by either blockade of macrophages Fc receptors or elution of immunoglobulin from the red cells suggests that the surface-bound immunoglobulin could be an important recognition signal for sickle cell destruction. While there is a reasonable consequence that increased immunoglobulin binding plays a role in the extravascular hemolysis of sickle cell disease, there is dispute regarding the membrane epitope involved in immunoglobulin binding. Proposed candidates are several, but the most convincing data suggest that clustering of membrane protein band 3 die creates oxidative denaturation of hemoglobin leads to accumulation of IgG and complement on the sickle cell surface. The findings of co-clustered band 3 and Heinz bodies and of increased surface immunoglobulin in both intake unmanipulated sickle red cells and oxidatively damaged normal red cells supports this thesis. A recent study of sickle cells that documented increasing constraints on the lateral and rotational mobilities of band 3 with increasing cell density tends further credence to the thesis of a cell density-dependent alteration in functional state of band 3 in sickle cells. Since thermodynamic considerations imply enhanced binding of autoantibody to band 3 in the clustered state compared with its normal state, it is likely that band 3 clustering is indeed a critical determinant of immunological recognition of sickle red cells. It should also be noted that the phagocytosis of the immunoglobulin-coated dense red cells by macrophages is greatly augmented by complement fixation on the membrane and that sickle cells have an impaired ability to inactivate surface complement.

Immunoglobulin-independent recognition of sickle cells by macrophages also has been postulated to account for extravascular hemolysis. Recognition of phosphatidylserine exposed on the outer half of the lipid bilayer as a result of loss of phospholipid asymmetry has been suggested as a signal. While this mechanism appears plausible, the data outlined to support this concept require further studies to evaluate this thesis critically. An important unanswered question relates to the relative contributions of immunoglobulin-dependent and immunoglobulin-independent recognition to erythrophagocytosis in vitro and to extravascular hemolysis in vivo.

Support for an important role of sickle red cell-macrophage interactions in red cell destruction is also found in clinical observations that show that the extent of sickle
cell adherence to macrophages correlates with the hemolytic index. Additionally, it has been noted that lower hematocrit are found in sickle patients in whom erythrophagocytosis by monocytes is evident in the peripheral blood. All of the data obtained to date are consistent with erythrophagocytosis being a major contributor to the hemolytic anemia of sickle cell disease.

In patients hospitalized during sickle cell painful crisis, there is a slight, brief decline in hemoglobin levels, followed by reticulocytosis. This decline in hemoglobin value does not appear to be due to transient aplastic crisis, since it is not accompanied by a decline in the number of reticulocytes. It suggests that physical entrapment and subsequent destruction of sickled red cells at sites of vasoocclusion may contribute to hemolysis during this phase of the disease. This may be particularly important in hypoxic vasculature sites where deoxygenation-induced sickling will generate rigid, dense, dehydrated cell. While there is no direct experimental evidence to support this thesis, a number of recent observations are consistent with this mechanism. It has been shown that during the period of acute pain, when hemoglobin values decline, the numbers of dense, rheologically compromised red cells in circulations also decrease. Furthermore, animal studies that examined the ex vivo flow behavior of human sickle red cells have shown that the rheologically compromised dense cells are selectively trapped in the vasculature. However, at the present time the potential mechanisms(s) responsible for the destruction of these trapped cells are unknown. The potential for hemoglobin polymer formation in various sickle cell disease genotypes has also been found to be related to the degree of anemia, suggesting a stronger relationship between polymerization tendency and hemolysis than that postulated for polymerization and clinical severity. Based on all of the currently available information, it is reasonable to suggest that trapping of rheologically compromised sickle red cells may in part be responsible for chronic and transient declines in hemoglobin level. The precise contribution of rheological abnormalities to red cell destruction warrants further study.
Increased levels of plasma hemoglobin observed in sickle cell patients indicate that intravascular hemolysis plays a role in the hemolytic process. Unfortunately, little is known about the mechanisms responsible for intravascular hemolysis. Recent studies, however, have provided some provocative insights into a potential mechanisms involving increased complement sensitivity of sickle red cell\textsuperscript{95,111}. It has long been known that repeated cycles of sickling and unsickling of cells results in shedding of membrane vesicles. The vesicles contain glycosylphosphatidylinositol (GPI)-anchored membrane proteins\textsuperscript{118}, including the complement regulatory proteins decay-accelerating factor (DAF) and membrane inhibitor of reactive lysis (MIRL, or CD59). Current data suggest that the increased complement sensitivity of sickle red cells stem from increased binding of the C5b-9 membrane attack complex, perhaps involving dysfunction of MIRL\textsuperscript{111}. Interestingly, the higher the cell density, the greater the extent of decrease in DAF and MIRL, but this is similar for sickle and normal cells\textsuperscript{111}. Thus, dense sickled red cells not only are phagocytosed by macrophages as a result of their ability to bind increased amounts of immunoglobulin and complement but also are abnormally sensitive to complement lysis.

Sickle red cells also exhibit increased susceptibility for mechanically induced cell fragmentation\textsuperscript{119}. In vitro studies have documented increased sensitivity of sickle red cells to fluid shear stress-induced mechanical fragmentation. The most dense cells are particularly susceptible to mechanical damage\textsuperscript{119}. The increased mechanical fragility of sickle red cells was most dramatically illustrated by the observation of red cell lysis in sickle cell patients undergoing vigorous exercise.

In addition to GPI-anchored proteins, sickle vesicles contain hemoglobin, glycophorins, and band 3\textsuperscript{118}. However, they exhibit a marked decrease in membrane skeletal proteins, including spectrin, ankyrin, protein 4.1, and actin. Membrane vesicles with similar biochemical characteristics have been isolated from whole blood samples of individuals with sickle cell disease, indicating that sickle red cells fragment in vivo as well, possibly from the same sickling-unsickling stress. Underlying membrane protein oxidation may confer increased susceptibility to this\textsuperscript{120}. During the process of membrane fragmentation, small quantities of hemoglobin are probably released into plasma. Also, the vesicles themselves contain hemoglobin, and
these are likely to be cleared rapidly from the circulation as a consequence of their unique surface properties. Thus, membrane fragmentation can contribute to decreased hemoglobin levels through shedding of hemoglobin-containing vesicles and through loss of hemoglobin into the plasma during the process of fragmentation.

3.4 APLASTIC CRISIS: Transient aplastic crises are due to a temporary cessation of red cell production in association with chronic hemolytic anemia. These aplastic crises are characterized by a decrease in the steady-state hemoglobin concentration, decreased reticulocytosis, and reduced red blood cell precursors in the bone marrow. The duration of aplastic crises is typically only a few days, but the degree of anemia usually is severe because the hemolytic process continues unabated without compensatory erythropoiesis. Aplastic crises have been recognized for many years in patients with hereditary spherocytosis and sickle cell anemia. It was known that these episodes occurred primarily in very young children with “viral symptoms”; they usually occurred only once in any given individual, and they occasionally were associated with an aplastic crisis in another affected family member at the same time. Taken together, these observations suggested that aplastic crises somehow are related to an infectious process.

The role of human Parvovirus (B19) in this process was first recognized in London when B19 antigen and/or antibody was detected in six children with sickle cell disease. Subsequently, there have been several confirmatory reports of erythroid hypoplasia occurring in sickle cell anemia patients in association with Parvovirus B19 in serum or the presence of IgM and/or IgG anti-B19 antibodies. Moreover, it now has been established that Parvovirus B19 is cytotoxic for actively cycling human erythroid progenitor cells. Taken together, these data support the concept that Parvovirus B19 is a major cause of transient aplasia in children with sickle cell anemia, accounting for 68 percent of cases (34 of 53 patients) form one institution over a 7 year period.

Infection with B19 during childhood and adolescence is relatively common since antibodies to human parvovirus are present in a majority of healthy adults. Aside from transient aplastic crises occurring in association with chronic
hemolytic disorders, a variety of other clinical problems are seen with B19 infection\textsuperscript{131}. Of note, the pediatric syndrome of transient erythroblastopenia of childhood, a pure red cell aplasia occurring in young children without an underlying chronic hemolytic disorder, is not related to Parvovirus B19 infection\textsuperscript{132}.

Whether Parvovirus B19 infection is responsible for hypoplastic erythroid crises in adults to the same extent as in children is not known. Clearly, other causes of aplastic crises also occur. There are reports of anemia and reticulocytopenia occurring in adults with sickle cell anemia with bone marrow necrosis\textsuperscript{133}. Of interest, however, two recent reports of adults with aplastic crises due to bone marrow necrosis also were associated with parvovirus infection\textsuperscript{134,135}. Another cause of aplastic crises in adults with sickle cell disease is related to oxygen inhalation therapy with secondary inhibition of erythropoietin production\textsuperscript{136,137}. In addition, aplastic crises may be due to other infections, including salmonella\textsuperscript{122}, streptococci\textsuperscript{124}, pneumococci\textsuperscript{124,127}, and Epstein-Barr virus\textsuperscript{127}.

Management of hypoplastic crises depends on the degree of anemia and cardiopulmonary distress. Since the magnitude of anemia during red cell aplasia generally is proportional to the severity of underlying hemolysis, transfusions are needed in sickle cell anemia more often than in Hb SC disease\textsuperscript{128}. If red cells are administered, usually only one transfusion is required since reticulocytosis occurs spontaneously within a few days of presentation.

3.5. Sickle Cell Hepatopathy: Sickle cell hepatopathy encompasses a range of hepatic pathology arising from a wide variety of insults to the liver in patients with sickle cell disease. It occurs predominantly in patients with homozygous sickle cell anemia, and to a lesser extent in patients with sickle cell trait, Hb SC disease and HbS b thalassemia. The hepatic disease may primarily be caused by the sickling process, but more commonly arises as a consequence of the multiple transfusions that these patients require in their lifetime. The term multitransfusion hepatopathy may therefore be more appropriate for these latter patients. The direct manifestations of sickle cell disease in the liver relate predominantly to vascular occlusion with acute ischemia, sequestration, and cholestasis, although chronic cholestatic syndromes have also been described. The main hepatic complications of multiple transfusions include acute and
chronic infection with hepatitis B and C and iron overload. A further potential consequence of the chronic hemolysis is the development of pigment stones, with consequent cholecystitis and acute and chronic biliary obstruction from choledocholithiasis. Abnormal liver function tests are common in patients with sickle cell anemia, even in the absence of liver disease. Raised bilirubin levels, predominantly unconjugated, are universal in sickle cell patients due to chronic hemolysis.

Johnson et al (1985) found that 72 of 100 patients with sickle cell anemia had an isolated elevation of bilirubin, with no other clinical or laboratory evidence of liver disease. Bilirubin levels correlate with lactic dehydrogenase levels\cite{138} suggesting that variable levels found in patients are related to the degree of hemolysis and/or ineffective erythropoiesis rather than to disorders of Bilirubin transport or processing. Hemolysis also raises plasma aspartate transaminase (AST) levels, which therefore also correlate with lactic dehydrogenase levels\cite{138}.

Mohamed AO et al (1993)\cite{139} reported Plasma alanine transaminase (ALT) levels therefore more accurately reflect hepatocyte injury in sickle cell patients. Elevations in the serum alkaline phosphatase are commonly seen in patients with sickle cell anemia, particularly during pain crises. However, studies suggest that bone alkaline phosphatase is the major enzyme fraction contributing to this increase.

**3.5.1. ACUTE SYNDROMES**

Patients with sickle cell disease may present with an acute syndrome characterized by right upper quadrant abdominal pain and jaundice. The differential diagnosis includes acute sickle hepatic crises, sickle cell intrahepatic cholestasis, acute viral hepatitis, cholecystitis, and choledocholithiasis with common bile duct obstruction. These can usually be differentiated by a careful history, liver function tests, serologic tests for viral hepatitis, and hepatobiliary imaging studies.

**3.5.1.1 Acute Sickle Hepatic Crisis**

Acute sickle hepatic crisis occurs in approximately 10% of patients with sickle cell anemia.\cite{140-142} Patients commonly present with acute right upper quadrant pain, nausea, low grade fever, tender hepatomegaly, and jaundice. Plasma AST and ALT levels
seldom exceed 300 IU/L, although levels of 1,000 IU/L or greater have occasionally been reported, presumably because of more severe hepatic hypoxic injury. Serum bilirubin levels are usually less than 15 mg/dL. Cocaine use by sickle cell anemia patients can have potentially disastrous results, precipitating a severe crisis due to synergistic hypoxic injury from cocaine-induced vasospasm and from sickling. Concomitant cocaine hepatotoxicity has been described in a patient with sickle cell crisis, who subsequently developed hepatic failure, coagulopathy, and encephalopathy. ALT elevations were higher (18-fold) than would be expected from a simple sickle hepatic crisis. The patient’s bilirubin peaked at 37.5 mg/dL, and prothrombin time at 14.7 seconds. A transjugular liver biopsy showed focal areas of confluent necrosis and large areas of collapse. The patient recovered gradually with supportive care.

Leonard et al (1998) found that patients with sickle cell anemia have hyperzincuria and systemic zinc deficiency caused by increased renal loss of zinc, which may lead to zinc deficiency. A recent study of 104 patients with sickle cell disease indicated that 44% had low plasma levels of zinc. Zinc is a cofactor for ornithine transcarbamylase, a urea cycle enzyme and inhibition of the urea cycle with resultant hyperammonemia may occur with zinc deficiency. Deferoxamine therapy may also increase fecal losses of zinc. The hyperammonemia may theoretically contribute to encephalopathy in cirrhotic patients with sickle cell anemia. The hyperammonemia of sickle cell anemia patients is reversible and can be corrected by zinc therapy. Zinc therapy also appears to significantly decrease the number of sickle/pain crises in treated patients, and zinc deficiency should therefore be corrected if present. Zinc may also regulate copper absorption from the gastrointestinal tract, and enhanced copper absorption and increased ceruloplasmin levels may also be seen with zinc deficiency.

Yahaya IA (2012) conducted study regarding biochemical features of hepatic dysfunction in Nigerians with SCA and reported that serum total proteins and albumin levels were found to fall within the reference intervals in both the HbSS

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patients and the controls. However, the mean bilirubin (total and conjugated) levels and the activities of alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase were significantly higher (P<0.05) in the HbSSpatients than the control subjects.\textsuperscript{151}

**Kehindo MO et al (2010)** were studied liver enzyme and trace elements in acute phase of sickle cell anemia and reported During crisis in sickle cell anaemia, liver enzymes, as well as the trace elements of Cu\textsuperscript{++}, Zn\textsuperscript{++} and Mn\textsuperscript{++} are increased; levels of aspartate aminotransaminase are strongly correlated with those of ALT and ALP. Levels of liver enzymes do not appear to be related to those of the trace elements in painful sickle cell crisis.\textsuperscript{152}

**Ebert EC et al (2010)** reported The most common laboratory abnormality is an elevation of unconjugated bilirubin level. Bilirubin and lactate dehydrogenase levels correlate with one another, suggesting that chronic hemolysis and ineffective erythropoiesis, rather than liver disease, are the sources of hyperbilirubinemia. Abdominal pain is very common in SCD and is usually due to sickling.\textsuperscript{153}

**Oparinde DP et al (2006)** conducted biochemical assessment regarding severity of sickle cell anemia with reference to role of hepatic enzyme and found that a significant increase in serum ALT, ALP and GGT levels in SCA with persistent hepatomegaly over those without hepatomegaly (p < 0.05, p < 0.05 and p < 0.01 respectively). All the index scores and the final aggregate severity scores were also significantly higher in SCA subjects with persistnt hepatomegaly. Only GGT demonstrated a fairly positive and significant correlation (r = 0.46, P < 0.05) with increased clinical severity among the hepatic enzymes.\textsuperscript{154}

**Kotila T et al (2005)** found that liver is one of the organs involved in the multiorgan failure that occurs in sickle cell disease, the pathophysiology of liver disease in this condition is complex because of the interrelated multifactorial causes. Liver dysfunction was assessed in both paediatric and adult sickle cell disease patients in the steady state. The mean (range) of Alanine transaminase (ALT),
Aspartate transaminase (AST) and alkaline phosphatase (ALP) were 23.0 (2-77) IU, 48.5 (15-120) IU, 227.5 (37-1200) IU respectively. ALT and AST levels were less than 100 IU in over 95% of the patients. The gender or age of the patients did not significantly affect the level of these three enzymes. There was close association between the liver size and elevation of the liver enzymes except for alkaline phosphatase (ALT=.017, AST=.009, ALP=.056). In conclusion minimal elevation of the transaminases which is not gender or age dependent were observed in steady state sickle cell disease, higher levels of alkaline phosphatase may be due to associated vasoocclusive crises involving the bones rather than a pathology of the liver.

Ojuwa et al (1994) studied thirty children with SCA and assayed serum alanine aminotransferase, alkaline phosphatase, total protein, albumin and bilirubin, during vaso-occlusive crisis and at recovery. Alanine aminotransferase, alkaline phosphatase and bilirubin levels were significantly higher during crisis than at recovery, (p < 0.005) especially in the young patient. However, the total protein and albumin levels were not significantly different in crisis and at recovery. A transient hepatic functional derangement during vaso-occlusive crisis is a probable explanation for the reported changes.

Isichei UP (1980) was studied 84 young homozygous sickle cell patients aged 1 to 11 and observed biochemical changes and reported no convincing evidence of liver cell damage except in 12% of cases. The normal transaminase observed in many of the patients assessed, together with the high alkaline phosphatase activity which seemed to be out of proportion to plasma bilirubin, is a picture compatible with localised obstructive lesions of the liver or bone lesions, both of which are common in sickle cell disease. This biochemical pattern suggests that the conjugated bilirubin, which dominates the picture in 40% of patients who have 'haemolytic jaundice', is due largely, not to liver cell damage, but to a combination of two factors, namely, intrahepatic cholestasis and the presence of actively functioning liver cells. Adequate
albumin synthesis found in these patients, together with normal thymol reactions, provides further evidence of the absence of severe liver cell damage.\(^{157}\)

**Brody JI et al (1975)** studied behavior of ALP in sickle cell anemia patients and found physical and biochemical criteria identified bone alkaline phosphatase as the principal, although not necessarily the sole, enzyme fraction that increases during symptomatic sickle cell crises. Moreover, there appeared to be concordance between crisis severity, serum levels of alkaline phosphatase, and isoenzyme patterns; electrophoretic and biochemical abnormalities could be detected even when the patients were asymptomatic. The present data suggest that the serum alkaline phosphatase level may be an additional indicator of the degree, frequency, and persistence of tissue injuries that occur in sickle cell anemia\(^{158}\).

**3.6. SICKLE CELL NEPHROPATHY**: Sickle cell nephropathy is now a well-characterized entity with specific manifestations, risk factors, and prognosis. The presence of renal failure in sickle cell disease (SCD) ranges from 5 to 18% of the total population of SCD patients\(^{159}\). Powars et al (1991)\(^{160}\), in a prospective, case-control study of patients with SCD compared with sickle cell hemoglobin C patients, documented 31 (4.2%) patients affected by renal failure. The median age at the time of renal failure was 23.1 yr. Survival time was 4 yr with a median age of death of 27 yr after the diagnosis of end-stage renal disease (ESRD) in spite of dialysis treatment. Proteinuria, hypertension, severe anemia, and hematuria were reliable predictors of chronic renal failure\(^{232}\). Recently, in a prospective survival analysis of 964 patients with sickle cell anemia in adults, Platt et al. (1994)\(^{161}\) observed an 18% overall mortality in adult SCD patients with 40% of these (7.6% of the total) manifesting overt renal failure. None received a kidney transplant. By multivariate regression analysis, renal failure was identified as the major risk factor for early mortality in adult patients with SCD. Sklar et al. (1990)\(^{162}\), in a population study of 368 patients, found chronic renal insufficiency in 4.6% of SCD patients that was significantly associated with proteinuria and increased age. In patients with SCD, priapism episodes\(^{163}\), especially those with postpubertal presentation and tricorporal disease (corpora cavernosa and
corpus spongiosa involvement), had increased risk of multiorgan failure, including kidney failure. Although there is no gender predilection for renal failure in most series, Nissenson and Port (1989)\textsuperscript{164} analyzed and reported a marked male predominance of sickle cell nephropathy (SCN) patients in the U.S. Renal Data System database, in which few patients were offered transplantation.

3.6.1. Pathophysiology

Chronic sickling underlies several mechanisms for kidney injury. The arterial side of the renal microvasculature has a low O\textsubscript{2} tension. The hypertonicity and low pH of the renal medulla promote the formation of hemoglobin polymers in the red cells with deformation of the sickled cells, resulting in an increase in the blood viscosity, functional venous engorgement, and interstitial edema, predisposing the renal microcirculation to ischemia and infarction\textsuperscript{165}. Obliteration of the medullary vasculature produces segmental scarring and interstitial fibrosis (structural papillectomy), resulting in the formation of dilated renal pelvic capillaries and veins. Hematuria may result from rupture of vessels from the early venous engorgement or from the dilated vessels that result from scarring. The development of collateral vessels and their abnormal orientation in the medulla interferes with the countercurrent exchange mechanism, culminating through the years in irreversible loss of medullary tonicity\textsuperscript{165}. Renal cortical blood flow and GFR are increased perhaps by the secretion of medullary vasodilator prostaglandins. Hyperfiltration coupled with glomerular hypertrophy can lead to glomerulosclerosis\textsuperscript{165, 166, 167}. Once progression of the glomerular damage is evident, GFR begins to decrease, likely with some contribution from the ingestion of analgesics that can independently induce interstitial nephropathy\textsuperscript{159}. Recently, Guasch \textit{et al.} (1997)\textsuperscript{167} documented a pattern of an increased dextran permeability in the glomerular basement membrane of SCD patients, with an incremental increase in the pore radius. This would cause a nonselective proteinuria rather than the microalbuminuria associated with hyperfiltration. Bank \textit{et al.} (1996)\textsuperscript{168} showed that in a transgenic sickle cell mouse model, there is an induction of nitric oxide synthase II (NOS II) in the glomeruli and distal nephron. This enzyme may increase the synthesis of nitric oxide leading to vasodilation and contribute subsequently to hyperfiltration.
Functional changes occur with increasing age in patients with SCD. In children and young adults there are increases in effective renal blood flow (ERBF), effective renal plasma flow (ERPF) and glomerular filtration rate (GFR), although the filtration fraction is decreased (Hatch et al., 1970). With age, there is a progressive decline in ERBF, ERPF and GFR and in patients over the age 40 years; GFR and ERPF tends to decline (Morgan and sergeant, 1981). But normal or above normal values may persist in some patients (Alleyne et al., 1975). Progressive renal failure at older ages is a major cause of illness and death (Morgan et al., 1987). Glomerular disease is common (15 – 30 percent) in homozygotes for sickle cell disease. Glomerular hyperfiltration and hypertrophy occur within the first 5 years of life. Approximately 15 – 30% of patients develop proteinuria in the first three decades, and 5% develop ESRD. The glomerular pathology is usually focal segmental glomerulosclerosis, probably due to sustained glomerular capillary hypertension or membrane proliferative glomerulonephritis (MPGN). Predictors of chronic renal failure are worsening anaemia, proteinuria, nephrotic syndrome and hypertension (Powers et al., 2005).

3.6.2 Sickle cell disease and glomerulopathy.
Patients with sickle cell anaemia (SCA) may develop glomerulopathy with proteinuria and progressive renal insufficiency leading to End Stage Renal Disease (ESRD) (Gausch et al., 2006). These authors observed that the patients with sickle cell haemoglobin (Hb SS) have a more severe disease than individuals with other sickling haemoglobinopathies using clinical, haematologic and biochemical parameters in a group of patients with sickle cell haemoglobinopathies. It was reported that increased albumin excretion rate (AER) occurs in 68% of the patients; macroalbuminuria was present in 26% and microalbuminuria in 42% while only 32% of adults with 'SS' disease had normoalbuminuria. There was no gender differences reported in the prevalence of albuminuria. In a study of proteinuria among SCA patients in Nigeria, male predominance of sickle cell nephropathy was reported (Abdu et al., 2011). The concentration of 24 hours urine protein in the SCA male subjects with proteinuria was significantly higher (0.25 g/day; p<0.001) compared with the SCA female patients with proteinuria (0.09 g/day) (Emokpae et al., 2010). The sex differences in the
mechanisms underlying renal injury suggest that androgens may permit or accelerate renal damage while estrogen may provide renoprotection (Ji et al., 2005)\textsuperscript{177}. The female sex hormone (estradiol) is thought to have antioxidant properties. Estradiol is capable of increasing superoxide dismutase and glutathione peroxidase expression as well as decreases NADPH oxidase enzyme activity and superoxide production (Lopez-Ruiz et al., 2008)\textsuperscript{178}. The graded albuminuria according to age hence duration of disease showed that, in ‘SS’ disease the prevalence of abnormal AER increased from 61% in patients aged 18 to 30 years to as high as 79% in patients older than 40 years. Albumin excretion rate was reported to have increased as creatinine clearance decreased, but there was a large variability and a significant number of patients had increased AER despite a preserved creatinine clearance. In a four decade observational study of 1056 patients with sickle cell disease, Powars \textit{et al},(2005)\textsuperscript{173} reported that 73% of the patients had one or more clinically recognized forms of irreversible organ damage. By the fifth decade, nearly one-half of the surviving patients (48%) had documented irreversible organ damage. ESRD (glomerulosclerosis), chronic pulmonary disease with pulmonary hypertension, retinopathy and cerebral micro infarctions were manifestations of arterial and capillary microcirculation obstructive vasculopathy. In an earlier report on chronic renal failure in sickle cell disease: risk factors, clinical course and mortality indicated that histologic studies showed characteristic lesion of glomerular “drop out” and glomerulosclerosis, in thirty six patients with sickle cell disease who developed sickle cell renal failure (Powards \textit{et al}, 2002)\textsuperscript{179}. Renal insufficiency in SCA was defined as a creatinine clearance <90ml/min using Crockcroft- Gault, (1976) equation. It was reported that 21% of patients with SCA had renal insufficiency while 27% of patients with other sickling disorders also had renal insufficiency but the percentage of patients with renal insufficiency and advanced kidney failure (chronic kidney disease stage 3 or higher) was higher in SS disease than other sickling disorders (Guasch \textit{et al},, 2006)\textsuperscript{174}. Guasch \textit{et al} (1997)\textsuperscript{180} previously showed renal insufficiency in SCA results from a glomerulopathy, which can be detected by the presence of albumin and other large molecular weight proteins in urine. Recently it was observed that glomerular involvement is extremely common in Nigerian sickle cell haemoglobinopathies (Abdu
Functional changes occur with increasing age in patients with SCD. In children and young adults there are increases in effective renal blood flow (ERBF), effective renal plasma flow (ERPF) and glomerular filtration rate (GFR), although the filtration fraction is decreased (Hatch et al., 1970)\(^{169}\). With age, there is a progressive decline in ERBF, ERPF and GFR and in patients over the age 40 years; GFR and ERPF tends to decline (Morgan and sergeant, 1981)\(^{170}\). But normal or above normal values may persist in some patients (Alleyne et al., 1975)\(^{171}\). Progressive renal failure at older ages is a major cause of illness and death (Morgan et al., 1987)\(^{172}\). Glomerular disease is common (15 – 30 percent) in homozygotes for sickle cell disease. Glomerular hyperfiltration and hypertrophy occur within the first 5 years of life. Approximately 15 – 30% of patients develop proteinuria in the first three decades, and 5% develop ESRD. The glomerular pathology is usually focal segmental glomerulosclerosis, probably due to sustained glomerular capillary hypertension or membrane proliferative glomerulonephritis (MPGN). Predictors of chronic renal failure are worsening anaemia, proteinuria, nephrotic syndrome and hypertension (Powars et al., 2005)\(^{173}\).

3.6.2 Sickle cell disease and glomerulopathy. Patients with sickle cell anaemia (SCA) may develop glomerulopathy with proteinuria and progressive renal insufficiency leading to End Stage Renal Disease (ESRD) (Gausch et al., 2006)\(^{174}\). These authors observed that the patients with sickle cell haemoglobin (Hb SS) have a more severe disease than individuals with other sickling haemoglobinopathies using clinical, haematologic and biochemical parameters in a group of patients with sickle cell haemoglobinopathies. It was reported that increased albumin excretion rate (AER) occurs in 68% of the patients; macroalbuminuria was present in 26% and microalbuminuria in 42% while only 32% of adults with ‘SS’ disease had normoalbuminuria. There was no gender differences reported in the prevalence of albuminuria. In a study of proteinuria among SCA patients in Nigeria, male predominance of sickle cell nephropathy was reported (Abdu et al., 2011)\(^{175}\). The concentration of 24 hours urine protein in the SCA male subjects with proteinuria was significantly higher (0.25g/day; \(p<0.001\)) compared with the SCA female patients with proteinuria (0.09g/day) (Emokpae et al., 2010)\(^{176}\). The sex differences in the
mechanisms underlying renal injury suggest that androgens may permit or accelerate renal damage while estrogen may provide renoprotection (Ji et al., 2005)\textsuperscript{177}. The female sex hormone (estradiol) is thought to have antioxidant properties. Estradiol is capable of increasing superoxide dismutase and glutathione peroxidase expression as well as decreases NADPH oxidase enzyme activity and superoxide production (Lopez-Ruiz et al., 2008)\textsuperscript{178}. The graded albuminuria according to age hence duration of disease showed that, in ‘SS’ disease the prevalence of abnormal AER increased from 61\% in patients aged 18 to 30 years to as high as 79\% in patients older than 40 years. Albumin excretion rate was reported to have increased as creatinine clearance decreased, but there was a large variability and a significant number of patients had increased AER despite a preserved creatinine clearance. In a four decade observational study of 1056 patients with sickle cell disease, Powars et al.,\textsuperscript{(2005)}\textsuperscript{173} reported that 73\% of the patients had one or more clinically recognized forms of irreversible organ damage. By the fifth decade, nearly one-half of the surviving patients (48\%) had documented irreversible organ damage. ESRD (glomerulosclerosis), chronic pulmonary disease with pulmonary hypertension, retinopathy and cerebral micro infarctions were manifestations of arterial and capillary microcirculation obstructive vasculopathy. In an earlier report on chronic renal failure in sickle cell disease: risk factors, clinical course and mortality indicated that histologic studies showed characteristic lesion of glomerular “drop out” and glomerulosclerosis, in thirty six patients with sickle cell disease who developed sickle cell renal failure (Powards et al., 2002)\textsuperscript{179}. Renal insufficiency in SCA was defined as a creatinine clearance <90ml/min using Crockcroft- Gault, (1976) equation. It was reported that 21\% of patients with SCA had renal insufficiency while 27\% of patients with other sickling disorders also had renal insufficiency but the percentage of patients with renal insufficiency and advanced kidney failure (chronic kidney disease stage 3 or higher) was higher in SS disease than other sickling disorders (Guasch et al., 2006)\textsuperscript{174}. Guasch et al.(1997)\textsuperscript{180} previously showed renal insufficiency in SCA results from a glomerulopathy, which can be detected by the presence of albumin and other large molecular weight proteins in urine. Recently it was observed that glomerular involvement is extremely common in Nigerian sickle cell haemoglobinopathies (Abdu
et al., 2011)\textsuperscript{175}. Increased AER occurs in approximately 70% of adults with haemoglobin SS disease and about 40% in adults with other sickling disorders. There was an indication of sickle cell glomerulopathy in a majority of older adults with SS disease and its prevalence was much higher than previously reported on the basis of a positive urinary dipstick for protein (Falk \textit{et al.,} 1992)\textsuperscript{181}.

Silva Junior GB \textit{et al.} (2012) were carried out renal function test in a cohort of 98 patients with sickle cell disease and reported Patients with glomerular hyperfiltration tended to be younger, had higher levels of hematocrit, hemoglobin and platelets and lower levels of urea and creatinine, with less frequent urinary abnormalities\textsuperscript{182}.

Bolarinwa RA \textit{et al.} (2012) were conducted study on renal disease associated with SCD observed that renal abnormalities, importantly albuminuria, is common in adult Nigerians with SCA and the pattern and incidence are similar to those reported from other parts of the world. Regular blood pressure monitoring, early diagnosis and active intervention are advocated to delay progression to end-stage kidney disease in view of poor outcomes of renal replacement therapy in SCA patients with nephropathy\textsuperscript{183}.

Arogundade FA \textit{et al.} (2011) were assessed kidney dysfunction and its risk factors in patients with SCD concluded that kidney disease is a common complication of SCD and significantly contributes to mortality. The age of the patients, duration of SCD and frequency of crises/hospitalizations are strong predictors of development of kidney disease\textsuperscript{184}.

Abdu A \textit{et al.} (2011) were reported after study of renal function in two hundred SCD patients that Proteinuria which is a marker of renal insufficiency is common among adult SCA patients, and routine screening for proteinuria may help detect those at increased risk of renal disease. CKD prevalence is high among SCA patients with significant proteinuria\textsuperscript{175}. 

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Aleem A (2008) was studied renal abnormalities in patients with sickle cell anemia and concluded renal abnormalities are present in a significant number of Saudi patients with SCD and proteinuria is the most common abnormality. Serum creatinine may remain low or within low-normal range in SCD patients despite reduced creatinine clearance. As proteinuria is a risk factor for developing renal failure in future, routine screening of SCD patients is recommended for timely intervention in order to prevent or delay renal damage\textsuperscript{185}.

Marsenic O et al (2008) reported early glomerular selectivity damage in children with SCD, which is secondary to both size-selectivity and charge-selectivity impairment. Microalbuminuria alone does not adequately detect early renal damage in children with SCD. Proximal tubular dysfunction is seen in younger children and is independent of glomerular damage. They suggested that children with SCD be tested for both total protein and IgG excretion in the urine in addition to albumin. Knowing the extent and type of renal damage may allow earlier recognition of renal injury and prompt earlier initiation of preventive therapies\textsuperscript{186}.

Marouf R et al (2006) were studied and compared cystatin C, beta(2)-microglobulin and creatinine as a renal marker and found that markers of GFR show variable ability to identify hyperfiltration in patients with SCD, but cystatin C is the best endogenous marker. Proteinuria is associated with age, haemoglobin, and abnormalities of GFR\textsuperscript{187}.

Bayzit Ak (2002) reported Mean serum creatinine, sodium, phosphorus and calcium levels were not statistically different between SCD patients and controls. Mean serum potassium and uric acid levels were significantly higher in patients than in controls. Thus concluded significant proximal tubular dysfunction is not a common feature but distal tubular abnormality is the most consistent renal functional derangement of patients with SCA in childhood\textsuperscript{188}.  

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Al-Naama LM et al (2000) conducted a study to determine the level of various biochemical parameters and reported that raised serum uric acid levels were found in Iraqi children with sickle cell disease. Creatinine clearance studies will be valuable to assess renal function\textsuperscript{189}.

Morgan AG et al (1984) found that renal insufficiency is common in adults with homozygous sickle cell disease. In a study consisting of 64 patients, serum urate concentration was dependent on renal urate clearance and also on creatinine clearance. The relation between serum urate and creatinine clearance was abnormal in patients with sickle cell disease and it is suggested that this might be caused by high single nephron glomerular filtration rates. Both the amount of urate excreted per millilitre of glomerular filtrate and the fractional excretion of urate increased with falling creatinine clearance, suggesting that the ability to increase tubular urate secretion was preserved. Patients with extensive tubular disease as shown by tubular proteinuria had serum urate concentrations which were not significantly different from those of age and sex matched non-proteinuric patients. Evidence that renal tubular disease interferes with urate secretion and causes hyperuricaemia in patients with sickle cell disease needs to be reinterpreted in the light of these findings\textsuperscript{190}.

Morgan AG & Serjeant GR (1981) studied 25 patients aged 40-60 with homozygous SS disease and reported that a possible mechanism of renal insufficiency in SS disease is cortical scarring, which is asymptomatic, not associated with hypertension, and accompanied by only minor proteinuria. A falling haemoglobin concentration is a sensitive and early indicator of renal impairment in SS disease\textsuperscript{191}.

3.7. Role of Cytokine in Sickle Cell Anemia:

Several cytokines, such as interleukin-1 beta (IL-1\beta) and tumor necrosis factor-alpha (TNF-\alpha), are associated with the activation of leukocytes, particularly monocytes and neutrophils, in SCA. Several other cytokines are also involved in the chronic inflammatory state that is present in SCA. The activation of cells and the release of cytokines stimulate the NF-\kappaB transcription factor pathway, which regulates...
the production of interleukin-4 (IL-4), interleukin-6 (IL-6) and interleukin-8 (IL-8). IL-6 and IL-8 production are also enhanced by the STAT3 intra-cellular pathway and proinflammatory activities\(^{228}\). Recently, the involvement of several other cytokines, such as IL-18, IL-17, IL-23, IL-12 and IL10, in inflammatory responses in SCA patients has been described. Because of the extensive participation of cytokines in the inflammatory processes involved in SCA pathology.

The interleukin-6 cytokine family promotes a variety of cellular functions, including differentiation, maturation, proliferation and survival. These cytokines are defined by their common usage of the widely expressed signal-transducing b subunit of the transmembrane receptor glycoprotein 130 (gp 130), which is a member of the class of type I cytokine receptors\(^{230,231}\).

The pro-inflammatory cytokine TNF-\(\alpha\) is produced mainly by monocytes/macrophages, but other cells, such as T-cells, smooth muscle cells, adipocytes and fibroblasts, can also produce this cytokine. TNF-\(\alpha\) is named for its ability to stimulate tumor necrosis and regression in vivo\(^{231}\). Biological responses to TNF-\(\alpha\) are mediated by two groups of receptors, TNFR55 and TNFR 75, which are present on the membrane of several types of cells, excluding RBCs\(^{232}\). Gene expression profiling in AA CD34+ cells has shown that more than half the upregulated genes are related to the immune response, including genes for cytokines and cytokine receptors, signal transduction genes, as well as other immune response genes. Many apoptosis and cell death genes are upregulated and some anti-apoptotic genes downregulated.

It is unclear why T cells are activated in AA. HLA-DR2 and its split HLA-DR15 and DRB1*1501 and 1502 alleles are overrepresented in AA, as a class I HLA-B*4002 and HLA-A*0206, indicating a possible role for antigen recognition. There is correlation between ATG response and DRB1*1501, but most of these HLA data come from studies of Japanese patients. Studies of cytokine gene polymorphisms that may reflect a heightened immune response in AA are limited; polymorphisms in the TNF-\(\alpha\) promoter, IL-6 and IFN-\(\gamma\) genes have been reported, but a systematic assessment of all potentially relevant cytokine genes has not been reported.
The specific cytotoxic T-cell targets on haemopoietic stem and progenitor cells in AA have not been identified. Potential candidates, identified by screening antibodies in patients' serum against a peptide library using leukaemia cell lines, include kinectin, DRS-1 (diazepam-binding inhibitor-related protein-1), PMS1 (postmeiotic segregation increased 1), moesin and hnRNPK (heterogeneous nuclear ribonucleoprotein K). However, the relevance of these findings is unclear, and they may represent epiphenomena rather than primary targets of cytotoxic T-cell attack.

Alternatively, a defect in the glycosylphosphatidylinositol (GPI) anchor may be the trigger for the immune response against normal progenitor cells through aberrant expression of intracellular GPI-protein(s) while at the same time providing a protective mechanism for GPI-defective cells. The expansion of PNH clones in a subset of patients with AA and hypoplastic MDS is also a well-established finding but the mechanism(s) that leads to this expansion is poorly understood. PNH cells may show a selective clonal advantage because (i) GPI-negative haemopoietic stem cells are spared from autoimmune attack because the absent GPI anchor is the target of the immune attack; (ii) GPI-negative cells acquire a second mutation that confers a survival advantage; or (iii) PIGA mutation are present in the blood of most healthy individuals but do not result in disease. They may also appear following treatment with the monoclonal antibody alemtuzumab, which recognizes the GPI-linked antigen CD52. ATG contains high levels of antibodies to the GPI-deficient proteins CD52 and CD48 and low levels of antibodies to CD16, which may contribute to the emergence of PNH clones after treatment of AA.