Assessment of $CD4^+$Tcells, $CD8^+$ Tcells and Macrophages in pruritic skin in polycythemia vera

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Polycythemia vera

Introduction:

Polycythemia vera (PV) is one of the chronic myeloproliferative disorders, which are collectively characterized by clonal proliferation of myeloid cells with variable morphologic maturity and hematopoietic efficiency. Polycythemia vera is distinguished clinically from the other chronic myeloproliferative disorders by the presence of an elevated red blood mass (RBM) (**Tefferi, 2005**).

Turk, in 1904, called attention to the occurrence of leukocytosis as well as to immature forms of cells of the red and white series, suggesting a hyperplastic disorder of blood formation involving the marrow as a tissue and not merely erythrocytes (**Turk, 1904**). In 1951, Dameshek drew attention to the relationship between four diseases PV, essential thrombocythemia, chronic myeloid leukemia, and chronic idiopathic myelofibrosis (CIMF) based on their common clinicopthologic features (**Dameshek, 1951**).

In 1967, the Polycythemia Vera Study Group (PVSG) was organized to identify the optimal approach to the diagnosis and treatment of PV (**Wasserman, 1971**).

Synonyms less commonly used for PV include erythremia, splenomegalic,Vaquez disease, Osler disease, myelopathic polycythemia, erythrocytosis megalosplenica and cryptogenic polycythemia (**Means, 2004**).

Epidemiology

A comprehensive review reported the incidence of PV to be 2.3 per 100.000 persons per year. The median age of patients diagnosed with PV is 60 years, although it can occur in person in all age groups (**Tefferi, 2003**). Polycythemia vera disease is very rare in children, as only about 30 patients have been described. It is also assumed that only 5% of the patients are younger than 40 years at diagnosis (**Girodon et al., 2005**).

Males have a slightly higher incidence with a male-female ratio of 1:1 to 2:1 (**Jaffe et al., 2001**). Familial cases have been documented, but their significance remains uncertain (**Gassas et al., 2005**). The disease is more common in Jews, especially Ashkenazi Jews (**Chaiter et al., 1992**). In a few cases, true PV may be familial, but apparently familial erythrocytosis is more often a result of a shared high-oxygen-affinity hemoglobin or a common exposure (e.g., a residence at high altitude or cobalt intoxication) (**Jefferson et al., 2002**).

Pathogenesis:

In general, the cause of PV is unknown (**Means, 2004).** Polycythemia vera is characterized by increased sensitivity of committed hematopoietic cells to its primary humoral growth factor, erythropoietin.

In vitro, Epo-independent erythroid colony formation is found in PV (**Van and Shannon, 2004).** However, no mutations of EPO or its receptor have yet been identified in PV. Moreover, subsequent studies revealed that marrow and blood cells from patients with PV were hypersensitive not only to Epo ,but also to several other hematopoietic growth factors, including interleukin 3 (IL-3), stem cell factor (SCF), granulocyte-macrophage colony-stimulating factor (GM-CSF) and insulin-like growth factor-1 (IGF-1) (**Axelrad et al., 2000).**

The most significant discovery in PV pathogenesis is the presence of a specific mutation in the (JAK2) (**Skoda and Prchal, 2005**). A unique valine to phenylalanine substitution at position 617 (V617F) has been identified called JAK2V617F that leads to a permanently turned on signaling at the affected cytokine receptors **(Kralovics et al., 2005)** all of the previous published studies showed that the majority of patients with PV (65–97%) have the JAK2 V617F mutation**.** An in vitro study has demonstrated that the expression of the mutated JAK2 induced Epo hypersensitivity and Epo-independent survival of cultured cell lines (**James et al., 2005).**

Another study found that basophil count was increased in patients with JAK2V617F -positive myeloproliferative neoplasms, particularly in those with polycythemia vera and there is an association between an increased number of activated basophils in the circulation of PV patients and the complaint of aquagenic pruritus.

Basophils are implicated in immediate hypersensitivity reactions and anaphylaxis, and their granules contain several biogenic amines, including histamine, which might be involved in the pathogenesis of pruritus although no clear correlation of pruritus with plasma histamine levels in PV has been found. However, it is also possible that other mediators such as leukotrieneC4 or granzyme B might also be involved in the pathogenesis of pruritus (**Lisa et al., 2009).**
Furthermore, basophils can produce and release a vast array of cytokines, such as IL-4, IL-13 and IL-33, which facilitate recruitment and activation of other inflammatory cells (including neutrophils, eosinophils, and mast cells); therefore, PV basophils might not necessarily act as effector cells by themselves in causing pruritus. Overall, the results of that study indicated that PV basophils are constitutively activated and hypersensitive to IL-3, favoring a direct role of JAK2V617F mutation. They also lend support to the hypothesis that activated basophils contribute to pruritus in PV patients **(Lisa et al., 2009).**

A significant correlation between the severity of pruritus and high numbers of cutaneous mast cells has been observed. It has been suggested that prostaglandins (PGE2) and may be other mediators released from mast cells, triggered by local vascular responses, are an important factor in the pathogenesis of pruritus in PV **(Jackson et al.,1987).**

The genetic basis for PV is uncertain. Karyotypic abnormalities are found in about 10% to 20% of patients (**Pearson et al., 2000**).

Clinical features

The lack of specificity of symptoms may, in fact, contribute to the delay in diagnosis. Some patients may have no complaints and are discovered incidentally. PV is characterized by a striking, absolute increase in the number of red blood corpuscles and in total blood volume. The symptoms and signs of PV (table 1) can be attributed in large part to the expanded total blood volume and to the slowing of the blood flow as a result of increased blood viscosity (**Means, 2004**).

TABLE1
Signs and Symptoms of Polycythemia Vera(Tefferi,2003; Hoffman,2000)

More Common	Less Common
• Hematocrit level >52 % (0.52) in white men, >47 % (0.47) in blacks and women • Hemoglobin level >18 g/ dL (180 g /L) in white men, >16 g / dL (160 g / L) in blacks and women) • Plethora • Pruritus after bathing • Splenomegaly • Weight loss • Weakness • Sweating	• Bruising/epistaxis • Budd-Chiari syndrome • Erythromelalgia • Gout • Hemorrhagic events • Hepatomegaly • Ischemic digits • Thrombotic events • Transient neurologic complaints (headache, tinnitus, dizziness, blurred vision, paresthesias) • Atypical chest pain

Skin and mucous membranes:

The color of the face is not cyanotic but is rather "ruddy", as might be produced by severe sunburn or a profound blush. The face also often appears swollen. This redness may be so intense that it produces a startling appearance. The face, particularly the lips, cheeks, tip of the nose and ears, and neck shows this color, but the skin of the trunk usually is not particularly affected. The distal portions of the extremities exhibit these changes more than the proximal portions and may be more cyanotic. These findings are not unique to PV, but are also observed in patients with an elevated hematocrit from secondary erythrocytosis. Ecchymoses of various sizes are common as the disease progresses. Red spots or brownish pigmentations of the skin may be found and a great variety of skin lesions have been observed, including dry skin, eczema, acneiform or urticarial changes and urticaria pigmentosa. The mucous membranes may be a deep raspberry-red, and epistaxis or gingival bleeding may occur (**Means, 2004**).

A common complaint is intense itching after exposure to water "aquagenic pruritus". This may be the presenting symptom of PV (**Abdel-Naser et al., 1993**), and is reported in up to 70% of PV patients (**Du peloux-Menage and Greaves, 1995**).

Cardiovascular system

Hypertension is relatively common in patients with PV. It is unclear whether this reflects the increased incidence of hypertension in the middle aged and elderly, or is a consequence of increased blood viscosity. Certainly, improvement of blood viscosity by reduction of the red cell volume will aid in the control of blood pressure. Thrombotic events, in both the arterial and venous circulation, are very common in patients with PV. 50.5% of thrombotic events occurred in the arterial circulation and 38.5% in the venous circulation (**Gruppo Italiano Studio Policitemia, 1995).** Other abnormalities of the venous system include varicosities and phlebitis. Moderate or significant thickening of the peripheral arteries, coronary thrombosis, claudications without occlusion, arterial occlusion with gangrene, Raynaud's phenomenon and thromboangitis obliterans have been described in patients with PV **(Reisner et al., 1992).**

Respiratory system

Dyspnea on exertion is common. Chest radiographs often reveal prominent vascular markings. Pulmonary hypertension is reported in patients with PV and other myeloproliferative disorders (MPDs) (**Reisner et al., 1992; Nand and Orfei, 1994**).

Gastro-intestinal system

In addition to nonspecific gastrointestinal symptoms, such as feelings of fullness, thirst and constipation, patients with PV have an increased frequency of peptic ulcer, gastrointestinal bleeding, or thrombosis of mesenteric vessels.

Duodenal ulcer has been found in 8% in patients with PV. It has been suggested that these ulcers follow thrombosis in the vessels of the first part of the duodenum and are produced by digestion of the area of local

necrosis. Hepatomegaly is common and cirrhosis has also been reported **(Means, 2004)**.

Splenomegaly

Splenomegaly occurs in more than two-thirds of PV patients. The size of the spleen varies greatly in individual patients and occasionally may extend to the pelvic brim. It is usually hard and smooth. Patients may experience pain in the splenic region, and after infarction, a friction rub can be heard in this area. Engorgement of the spleen with blood and extramedullary hematopoiesis are the major contributors to splenomegaly **(Messinezy et al., 1997).**

Central Nervous System

Headache is the most common neurological symptom, but lassitude, vertigo, syncope, insomnia, weakness, sensation of fullness in the head and numbness in the fingers are common. Vascular lesions of the brain constitute the most serious complication of PV. Hemiparesis, seizures and alteration of the cerebral functions have been reported in patients with PV. All such symptoms and signs are secondary to increased blood volume and /or decreased blood flow. Central vascular events represent 30% of the thrombotic events (**Gruppo Italiano Studio Policitemia, 1995**).

Visual disturbances

Visual disturbances are common and include transitory diminution of vision or even temporary blindness, scotomas, bright points in front of the field of vision, diplopia and temporary paralysis of one of the eye muscles. On fundus examination, the vessels may be engorged and tortuous. Papilledema and embolism of the central retinal artery have been reported **(Means, 2004).**

Blood and laboratory findings:

Red Blood Cell Mass, Hemoglobin and Hematocrit:

Red blood cell (RBC) mass, one of the PVSG diagnostic criteria, is determined by comparing total blood volume to plasma volume (normal value in male: 24-32 ml/kg; female: 21-27 ml/kg). It was originally thought to be a sensitive marker for PV, but subsequent

studies reported a relatively low predictive value when several confirmed PV cases were demonstrated to have a RBC mass less than the reference limit **(Michiels, 2004; Tefferi et al., 2005)**. Hemoglobin concentration typically is in the range of 18 to 24 g/dl (normal value in male: 13.5-17.5 g/dl; female: 11.5-16 g/dl). Red cell counts of 7 to 10 x 10^{12}/L are common when the patients with the disease are first evaluated (normal value in male: 4.5-6 x 10^{12}/L; female: 3.5-5 x 10^{12}/L). The normal haematocrit(HCT) range for adult males is 40-50 %, and 36-46 % for adult females, the highest hematocrit recorded is 0.92 and a red cell count of 10.37 x 10^{12}/L (**Pearson and Messinezy, 2001**).The majority of female patients with hemoglobin concentration >16.5g/dl (or (HCT) above 50 %) and all male patients with a hemoglobin concentration >18.5 g/dl (or HCT above 56 %) have an increased RCM (**Ferrant, 1994**). Thus determination of RCM may be unnecessary in patients with hemoglobin or hematocrit exceeding the above noted values (**Fairbanks, 2000; Sirhan et al., 2005; Tefferi et al., 2005; Johansson et al., 2005**).

White Blood cell (WBC) and Platelet Count:

Leukocyte counts of 25 x 10^9 /L are not uncommon, and values above 50 x 10^9 /L have been reported (**Pearson and Messinezy, 2001**).However, the total

WBC may not accurately reflect disease activity, since neutrophils and not lymphocytes or monocytes are increased in PV. Thus, newer criteria have replaced the total WBC count with an absolute neutrophil count > 10,000/ul (**Pearson and Messinezy, 1996; Michiels and Juvonen, 1997**). The platelet count is frequently increased, usually in the 500 to 1000 X 10^9 /L range, but counts as high as 6000 X 10^9 /L have been reported. Morphologic and qualitative functional platelet abnormalities often are detectable (**Van Genderen et al., 1995**). However, the leukocyte and platelet counts are not always increased above normal in patients with otherwise typical disease (**Means, 2004**).

Arterial oxygen saturation and carbon monoxyhemoglobin level:

The majority of PV patients have normal arterial oxygen saturation (SaO_2). Measuring SaO_2 and carboxyhemoglobin (COHB) levels is important to rule out hypoxia as a secondary cause for

erythrocytosis. A SaO_2 below 92% has been taken to indicate a causal relationship with an absolute erythrocytosis. Pulse oximetry is the most convenient method of measuring the SaO_2 **(Pearson et al., 2000).**

Serum erythropoietin:

In PV, the vast majority of patients express very low levels of serum Epo **(Messinezy et al., 2002; Mossuz et al., 2004**).Although the molecular mechanism responsible for low Epo level is not fully understood, it may be related to associated decrease in hemoglobin oxygen affinity **(Hess et al., 1994; Johansson et al., 2004).**

Serum ferritin; vitamin B_{12} and folate:

Vitamin B_{12} levels are elevated to more than 900 pg/ml in approximately 30% of patients, and 75% of patients show elevated unbound vitamin B_{12} binding capacity greater than 2200 pg/ml. This is because of increased transcobalamin-III, a binding protein found in white blood cells, and reflects the total white blood cell counts in the peripheral blood and bone marrow. Low ferritin values and folate deficiency have also been reported (**Pearson et al., 2000).**

Neutrophil alkaline phosphatase score:

A raised neutrophil alkaline phosphatase (NAP) score was taken as a diagnostic marker of PV by the Polycythemia Vera Study Group (PVSG). However, the technique is only semi-quantitative, liable to considerable inter-observer and inter-laboratory error, and there are no standardized controls (**Pearson et al., 2000).**

Other laboratory findings:

The erythrocyte sedimentation rate (ESR) is significantly increased. The basal metabolic rate may be moderately increased. The urine may be normal, albuminuria is found occasionally, and, less often, casts are present. The specific gravity of the blood is 1.075 to 1.080, compared to the normal range of 1.055 to 1.065. Hyperuricemia occurs in 40% of patients and reflects the high turnover rate of bone marrow cells releasing DNA metabolites. Hyperkalemia has been noted when platelets are greatly increased. Hyperhistaminemia and hyperhistaminuria were reported in patients with PV, which may explain, in part, the pruritus often present (**Tefferi, 2003).**

Bone marrow aspirate/trephine

Bone marrow morphology was not originally included in the PVSG diagnostic criteria for PV; several large histopathologic series examining patients who presented with mild to significant erythrocytosis demonstrated that PV can be differentiated from secondary polycythemia in approximately 96% of cases (**Michiels, 2004; Thiele et al., 2005**). An increased number of megakaryocytes in a moderately to markedly hypercellular marrow has been considered one of the hallmarks of PV **(Michiels, 2005; Thiele and Kvasnicka, 2005**).

Some fibrosis may be seen in 10% to 15% of patients (**Michiels et al., 2000**).

In the first PVSG study, 281 pretreatment bone marrow biopsies were obtained and the following observations were noted:

- The most common abnormality was the absence of stainable iron in 94 percent of patients.
- Cellularity varied from 36 to 100 % (mean 82 %, normal: 35 to 50 %).
- The numbers of megakaryocytes and amount of reticulin were variable, although both were generally increased (**Peterson and Ellis, 1995).**

Clonal markers:

Bone marrow examination can be also used to identify clonal markers that have been associated with PV; such as deletion of the long arm of chromosome 20, trisomy for chromosome 8 or 9 or loss of heterozygosity on the short arm of chromosome 9 which are found in up to 30 % of previously untreated patients with PV (**Andrieux and Demory, 2005**).

As mentioned before, 65 to 75 % of patients have shown the presence of a specific mutation in the Janus Kinase -2gene (JAK2) with valine to phenylalanin substitution at position 617 (JAK2V617F), which is absent in normal subjects as well as those with secondary polycythemia (**Tefferi et al., 2005**). Interphase fluorescent in situ hybridization on granulocytes has been shown to be useful in recognizing PV karyotypic abnormalities (**Pearson at al., 2000**).

Genetic testing:

Genetic testing can be used to rule out the possibility of chronic myeloid leukemia. Patients with this disease have a characteristic chromosomal abnormality called the Philadelphia translocation chromosome, which does not occur in patients with PV (**Tefferi, 2003**).

Imaging Studies:

Computerized tomography (CT) scanning or ultrasonography of the abdomen can be used to assess the size of the spleen, which is frequently enlarged in PV (**Messinezy et al., 1997**). Renal pathology, cerebellar hemangioblastoma, and pheochromocytomas that can cause secondary polycythemia may also be detected (**Means, 2004**).

Diagnosis:

The PVSG (1975) have developed a set of criteria that are useful clinically and conceptually (table 2).

Table (2): PVSG diagnostic criteria and their significance (Berlin, 1975)

Criteria	Significance
Major criteria (M) - M1. Increased RCM (males>36ml/kg and females >32ml/kg) - M2. Normal arterial oxygen saturation (SaO_2 >92%) - M3. Splenomegaly **Minor criteria (m)** - m1. Thrombocytosis (Platelets >400,000/ml) - m2. Leukocytosis (Leukocytes > 12,000/ml) - m3. NAP score >100 (no fever or infection) - m4. Serum vitamin B_{12} >900 pg/ml or unsaturated B12 binding capacity >2200 pg/ml.	- M1: Identifies actual polycythemia versus spurious polycythemia. - M2: Rules out the most common etiology of secondary polycythemia (arterial hypoxemia). - M3 or m1-m4: Evidence of a myeloproliferative state.

The diagnosis of PV requires the presence of all three major criteria (M) or the first two major criteria and two minor criteria (m). The first major criterion provides confirmation that the patient has actual polycythemia, as indicated by elevated red cell mass (RCM). The second major criterion rules out the most common etiology of secondary polycythemia, which is severe arterial hypoxemia, by demonstrating a normal SaO_2. The third criterion, which is evidence of a myeloproliferative syndrome, is provided either by the third major criterion, splenomegaly, or by two of the four minor criteria. It should be remembered that the PVSG's goal was to establish exclusive criteria, which would mean that all patients included in their protocols had clear-cut and inarguable PV. Although most of PV patients meet the diagnostic criteria, some do not (**Pearson and Messinezy, 2001).**

Table (3): Causes of secondary erythrocytosis (**Pearson and Messinezy, 2001**)

Physiologically appropriate:
- Chronic pulmonary or cardiac disease
- Decreased 2,3-diphosphoglycerate
- High oxygen affinity hemoglobinopathy
- Increased carboxyhemoglobin (in smokers) and methemoglobin
- Residence at high altitude
Physiologicallv inappropriate:
- Adrenal cortical hypersecretion
- Hydronephrosis
- Tumors producing erythropoietin or anabolic steroids
Relative (stress):
- Disorders associated with decreased plasma volume (e.g. diarrhea, emesis, renal diseases)

Treatment:

The aim of therapy in PV is to produce a reduction in the RCM by methods that:

- Permit the longest survival.
- Are associated with fewest significant complications, allowing

the patient maximum quality of life.

- Are least expensive and convenient for the patient.

(a) Phlebotomy:

The aim of phlebotomy is reducing the hyperviscosity of the blood by decreasing the venous haematocrit level to less than 45 percent in white men and 42 percent in blacks and women. The initial treatment for most PV patients is phlebotomy. In the plethoric phase, PV is treated first by performing phlebotomy until the haematocrit is under reasonable control. Most patients can tolerate removal of 450 – 500 ml of blood every 2-4 days. As more blood is removed and the patient becomes iron deficient, the haematocrit becomes easier to control, and the phlebotomy schedule should be adjusted accordingly. Although phlebotomy is effective for controlling erythrocytosis, it does not affect the variable leukocytosis and thrombocytosis. Many patients can be maintained in normal state by phlebotomy together with a few simple adjuvants, when necessary, to control hyperuricemia or pruritus (**Gilbert, 2001; Tefferi, 2003**).

The advantages of phlebotomy are that it is inexpensive, requires extremely limited technical support, offers prompt and effective reduction of the red cell mass and blood volume to normal values and allows rapid control of symptoms. The PVSG found a statistically significant increase in the number of thrombotic events within the first three years of initiating treatment, compared with the use of myelosuppressive agents. After this period, however, the rate of thrombosis remained the same for both treatment approaches. The PVSG reported the best median survival, 12.6 years, for this type of treatment (**Pearson et al., 2000**). The use of myelosuppressive agents such as radioactive phosphorus (^{32}P), chlorambucil (Leukeran), busulfan (Myleran), and hydroxyurea (Hydrea) in conjunction with phlebotomy has been studied. Chlorambucil and busulfan, all alkylating agents, have fallen out of favor because of concerns about rates of iatrogenic leukemia **(Barbui and Finazzi, 1998).** The agent ^{32}P remains in use with supplemental phlebotomy and has a reported median survival similar to that of phlebotomy alone-10.9 years according to PVSG data**(**Berk et al.,1995) and 11.8 years according to GISP 1995.The myelosuppressive drugs such as ^{32}P had an initial

advantage over phlebotomy alone regarding thrombosis rates during the first three years of treatment. However, this effect disappeared after three years, and rates of thrombosis thereafter were equivalent **(Berlin, 2002)**.

In summary, phlebotomy remains the cornerstone of therapy of PV, but additional myelosuppressive treatments are required in most patients (**Pearson et al., 2000**).

b)Antithromboticthерapy:

In patients with PV, the use of Acetyl Salicylic Acid (ASA) has long been controversial, mainly based on the results of the PVSG study. In that study, the administration of 900mg ASA plus 75 mg dipyridamole in combination with phlebotomy was compared randomly with ^{32}P. The significantly higher rate of severe gastrointestinal bleeding and thromboembolic complications observed in patients receiving ASA led to a discontinuation of the study. Since then, ASA in PV was considered to be a dangerous and obsolete management of PV (**Tartaglia et al., 1986**).

Later reports showed that inhibition of platelet aggregation with low-dose ASA reduced the rate of arterial and venous thromboses in patients with increased risk of cardiovascular complications. These results prompted the Italian PVSG to perform a randomized pilot study to investigate the safety of ASA (40 mg/d) in comparison with placebo in patients with PV. No increased risk of bleeding or other significant side effects were observed during the administration of ASA, leading to the conclusion that this dose of ASA is safe (**Gruppo Italiano Studio Polycythemia vera, 1997**). The pilot study was followed by a large study by European Collaboration of Low Dose Aspirin in Polycythemia Vera (ECLAP, 1997) to confirm the safety and to evaluate the efficacy of low-dose ASA in patients with PV. Five hundred and eighteen patients were randomly assigned to receive ASA (100 mg/d) or placebo. The results showed that low-dose ASA could safely prevent thrombotic complications (significant reduction of the risk of the combined end point of nonfatal myocardial infarction, nonfatal stroke, pulmonary embolism, major venous thrombosis, or death from cardiovascular causes) in patients with PV. However, the overall mortality was not reduced significantly and the risk of major bleeding

complications was not increased significantly in the ASA group (**Landolfi et al., 2004**).

C) Chemotherapy:

i. Alkylating agents:

A number of chemotherapeutic agents have been used for the treatment of PV. These include benzene, Fowler's Solution (Potassium arsenate), nitrogen mustard, melphalan, triethylene melamine, thiotepa, pyrimethamine, vercyte (a neutral piperazine compound), and a variety of other agents. All of these have fallen into disuse in PV, typically through a poor toxicity-to-efficacy ratio. Busulphan, and more recently, chlorambucil, are two alkylating agents that were widely used in PV, but the increased frequency of leukemia observed with alkylating agents led to a discontinuation of their routine use **(Means, 2004).**

ii. Hydroxyurea:

Hydroxyurea (HU) is a non-alkylating myelosuppressive agent that impairs DNA synthesis through the inhibition of ribonucleotide reductase (**Yarbro, 1992**). PVSG data have established this agent to be an effective bone marrow suppressant. HU is widely used in the treatment of PV, because it is less leukemogenic. The incidence of leukemia in patients treated long-term with HU appears to be low, and similar to that observed in patients treated with phlebotomy alone (**Michiels et al., 2000**). Patients carrying the acquired JAK2 V617F mutation have been demonstrated to obtain better cytoreduction and have a lower rate of arterial thrombosis when treated with hydroxyurea rather than anagrelide (**Campbell et al., 2005).** HU permits rapid reduction of leukocyte and platelet counts; supplemental phlebotomy may occasionally be necessary to reduce the red cell mass (**Berk et al., 1986).**

The adverse dermatological effects of HU have been well documented. These include xerosis, diffuse hyperpigmentation, brown nail discoloration, stomatitis, erythema, and scaling of the face, hands and feet (**Renfro et al., 1991**). Mucocutaneous ulcers have been a relatively frequently reported side effect of HU (**Vassallo et al., 2001**). Leg ulcers were reported to occur in 9% of patients taking HU medication and these ulcers were completely refractory to topical or