NovoSeven® Epotaxog alfa (activated) Abbreviated Prescribing Information: NovoSeven (Recombinant Coagulation Factor VIIa (rFVIIa)) Presentation: Powder for injection with accompanying solvent for reconstitution (Water for Injections). Available in packs containing 1.2, 2.4 or 4.8 mg rFVIIa. Uses: Treatment of bleeding episodes and prevention of bleeding during surgery or invasive procedures in patients with: congenital haemophilia with inhibitors to coagulation factors VIII or IX or XI or both or patients who have been exposed to a high dose of anti-FVIII inhibitors; acquired haemophilia—congenital FVIIa deficiency; Glanzmann's thrombasthenia with antibodies to GPIIb-IIIa and/or A IIa, with past or present refractoriness to platelet transfusion. Dosage: The rFVIIa is dissolved in the accompanying solvent before use. After reconstitution the solution contains 0.6 mg rFVIIa/ml. 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Repeat dose at 2-3 hour intervals for first 24-48 hours. In major surgery, continue dosing at 2-4 hour intervals for 6-7 days. Dosage interval may then be increased to 6-8 hours for further 2 weeks. Treatment may be up to 3 weeks until healing has occurred. Factor VIII deficiency: For bleeding episodes and for invasive procedures/surgery administer 15-30 µg/kg body weight every 4.6-6 hours until haemostasis achieved. Adjust dose and frequency to individual. Glanzmann's thrombasthenia: For bleeding episodes and for invasive procedures/surgery administer 50-90 µg/kg body weight every 4-6 hours until haemostasis achieved. Administer three doses with an interval of 2-3 hours. Haemophilia A or B with inhibitors or acquired haemophilia. Initial dose of 99 µg/kg body weight; dose every 2 hours until clinical improvement. At least three doses should be administered to secure effective haemostasis. For patients who are refractory platelets are first line treatment. 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52. The Father of Blood Banking
Paroxysmal nocturnal hemoglobinuria (PNH) is a rare, debilitating, and life-threatening hematologic disorder. Clinically, PNH is characterized by the classical triad of acquired Coombs-negative intravascular hemolytic anemia, thrombophilia, and bone marrow failure to various degrees.1-4

This clinical entity was first described in 1882 by the German physician Paul Strübing from Greifswald.5 In 1911, this form of hemolytic anemia was reported in conjunction with the characteristic hemoglobinuria (Figure 1) by Marchiafava and Micheli,6,7 which finally gave rise to the eponymous name Strübing-Marchiafava-Micheli syndrome.

In PNH, the absence of the glycosylphosphatidylinositol (GPI)-anchored complement inhibitory protein CD55 and most importantly CD59 from the surface of PNH red blood cells (RBCs) renders them susceptible to terminal complement-mediated lysis.8-10 This is a consequence of mutations in the phosphatidylinositol glycan-A gene resulting in a decrease in, or total deficiency of, GPI-anchored proteins.11,12

The gold standard for the diagnosis of PNH is flow cytometry with the measurement of GPI-anchored proteins as well as the GPI-anchor itself (FLAER) and has replaced the Ham test and the sucrose lysis test.3,13-16 Flow cytometry allows sensitive and specific detection and quantification of GPI-deficient populations in various cell lineages, and it can be used for diagnosis and monitoring during follow up. Ideally, at least the lack of 2 different GPI-anchored proteins on at least 2 different cell lineages should be used to diagnose PNH.1,17,18 Together with the assessment of hemolytic parameters and bone marrow investigation, including...
an aspirate, cytogenetics, and a biopsy, the minimal essential criteria for the diagnosis and categorization of PNH are met.1

The chronic intravascular hemolysis in PNH is the cause for weakness, pallor, fatigue, anemia, dyspnea on exertion, reduced quality of life, the need for transfusions, renal impairment, pulmonary hypertension, and the risk of life-threatening thromboembolic complications.19,20 Free hemoglobin leads to depletion of nitric oxide in plasma, causing complications associated with smooth muscle dystonias, including abdominal pain, dysphagia, pulmonary hypertension, and erectile dysfunction.1,19

Eculizumab (Soliris™; Alexion Pharmaceuticals, Incorporated, Cheshire, CT, USA) is a humanized monoclonal antibody that binds to the complement protein C5. Eculizumab inhibits the terminal complement cascade, thereby preventing complement-mediated destruction of PNH RBCs.21 Before the approval of eculizumab in March 2007 by the Food and Drug Administration in the USA and in June 2007 by the European Commission for the treatment of patients with PNH, therapeutic options were mainly directed towards palliation of symptoms rather than the underlying hemolytic process. With the availability of a targeted treatment in PNH, eculizumab has become the standard treatment for symptomatic PNH.

**How to treat patients with PNH**

**Historical treatment of PNH**

Historically, 2 treatment approaches were available in PNH, namely, symptomatic treatment and prophylaxis of complications or stem cell transplantation (SCT). However, with symptomatic treatment and prophylaxis, long-term control of the disease was rather unsatisfactory as well as SCT with the potential for cure but also a high treatment related morbidity and mortality due to infections, graft-versus-host disease GVHD as well as graft failure.1,3,22 Possible treatment options today are listed in Table 1 in detail.

Based on the pathophysiology of PNH, inhibition of the complement system was a rational and targeted approach.

**Inhibition of the terminal complement**

Eculizumab (Soliris®, Alexion Pharmaceuticals) is a first-in-class, humanized, monoclonal antibody directed against the terminal complement protein C5. The germine human framework acceptor sequences were used to minimize immunogenicity and the human IgG2/4 heavy chain constant regions to eliminate the ability of the antibody to bind Fc receptors and activate the complement.21,23 It has a very high binding affinity for human C5, and each molecule binds 2 C5 proteins. Hereby, terminal complement cascade with the formation of the membrane attack complex C5b-C9 is blocked by preventing its cleavage to C5a and C5b (Figure 2). Importantly, the generation of components in the early steps of complement activation remains intact, which are critical for immunoregulation and protection against infections.21,23

Results from the phase III, multicenter, double-blind, placebo-controlled TRIUMPH study demonstrated that eculizumab reduces hemolysis, transfu-
sion requirements, and improves fatigue in patients with PNH. The SHEPHERD study, an open-label, phase III, safety and efficacy trial that enrolled a more heterogeneous population of patients with PNH (including those with significant thrombocytopenia and minimal transfusion requirements) showed similar benefits of eculizumab. Based on its efficacy in those 2 phase III clinical trials, eculizumab was approved in the US and Europe for use in PNH in 2007. Eculizumab is highly effective in reducing intravascular hemolysis in PNH. However, it does not improve the associated bone marrow failure and mild hemolysis via extravascular sequestration of PNH RBCs loaded with C3 cleavage product becomes relevant. Therefore, eculizumab is the most effective in classical PNH patients as compared to PNH in the setting of another bone marrow failure syndrome, e.g., aplastic anemia (AA), myelodysplastic syndromes, or osteomyelofibrosis.

Eculizumab is administered intravenously over 35 minutes as an infusion independent of the body weight at a dose of 600 mg weekly for the first 4 weeks, then 900 mg biweekly starting on week 5 (Figure 3). Inhibition of the complement at C5 increases the risk for infections with encapsulated microorganisms, especially Neisseria meningitidis and N. gonorrhoeae. Thus, all patients should be vaccinated at least 2 weeks before the start of the eculizumab treatment, preferentially with a tetravalent, conjugated meningococcal vaccine. Patients should also be revaccinated every 3 to 5 years and be instructed about early clinical signs or symptoms of meningococcal infections and then seek immediate medical attention. The most common side effect reported during eculizumab treatment was headache, which occurred in approximately half of the patients and most of the time within the first 24 hours after the first dose or two. Otherwise, eculizumab is safe and well tolerated.

Treatment of PNH in the era of eculizumab
Eculizumab was approved for any patient diagnosed with PNH. However, eculizumab should preferentially be used for symptomatic PNH patients with severe fatigue, recurrent hemolytic crisis with abdominal pain, renal insufficiency, thromboembolic events, or other end-organ complications. PNH patients with no or only mild symptoms could be followed by watchful waiting. In some patients, the eculizumab dose must be adjusted due to breakthrough hemolysis. Typically, this occurs 1 or 2 days before the next scheduled dose together with a spike of the LDH level. This can be treated by either shortening of the interval from 14 to 12 days or increasing the eculizumab dose from 900 mg to 1200 mg biweekly. Bacterial infections can still trigger hemolytic crisis, even during treatment with eculizumab due to increased complement activation. Thus, an immediate and consequent antibiotic treatment in case of bacterial infections is recommended. In case of a hemolytic crisis, patients should be administered intravenous fluids to ensure hydration. Increased complement activation can also be triggered by viral infections, surgery, trauma, or pregnancy. Despite the effective eculizumab treatment, some PNH patients still require RBC transfusion based on symptoms of anemia.
Monitoring
A continuous monitoring remains essential in treated and untreated patients with PNH. I recommend monitoring of complete blood count, including reticulocyte count, LDH, and more thorough parameters of hemolysis (haptoglobin, hemopexin, and bilirubin). Determination of the reticulocyte production index is also helpful to accurately reflect the marrow production of erythrocytes. To follow renal function, creatinine levels should be measured. BNP levels can be a noninvasive parameter for pulmonary hypertension.

Elevated D-dimers are associated with activated coagulation and thrombosis. Even more, there is an increase in iron storage especially in PNH patients requiring blood transfusions. Routine supplementation of iron becomes obsolete, particularly in PNH patients requiring blood transfusions. In case of a relevant iron deficiency or iron overload (1), PNH clone size measurement in PNH patients with an otherwise stable disease is recommended yearly. In the situation of changes in clinical parameters, however, reevaluation should be done. In case of inadequate response on eculizumab treatment or continuous increase of transfusion requirements, a bone marrow investigation should be performed to rule out AA or clonal transformation.

After the start of the eculizumab treatment, intravascular hemolysis is reduced dramatically characterized by the LDH levels returning to normal or near normal within days to weeks. Reticulocytes often remain elevated as extravascular hemolysis becomes relevant as mentioned before. Screening for extravascular hemolysis by using the monospecific direct agglutination test is useful during eculizumab treatment. If clinically relevant, low dose corticosteroids are reported to be a treatment option.

Supplementation and depletion
As elevated reticulocytes indicate an increased regeneration along with an erythroid hyperplasia in the bone marrow, a sufficient supply with folic acid and vitamin B12 should be warranted. I generally recommend the daily intake of 1–5 mg folic acid and vitamin B12 if deficient. A significant urinary iron loss from hemoglobinuria and hemosiderinuria is common in hemolytic PNH patients with the need for oral iron supplementation. However, as eculizumab blocks intravascular hemolysis effectively, there is no further urinary iron loss. Even more, there is an increase in iron storage especially in PNH patients requiring blood transfusions. Routine supplementation of iron becomes obsolete, and in case of a relevant iron overload, iron depletion should be initiated.

Prevention and treatment of thrombosis
The most feared complications and the leading cause of death in PNH are thromboembolic events. Eculizumab, however, has clearly demonstrated in the clinical trials that long-term treatment significantly reduces the risk for thrombosis in PNH from 7.37 events/100 patient-years to 1.07 events/100 patient-years (85%, P<0.01). Interestingly, in the subgroup of patients already on antithrombotic treatment, the thromboembolic events were reduced by 94% from 10.6 events/100 patient-years before eculizumab treatment to 0.62 events/100 patient-years during eculizumab treatment (P<0.01).

In acute thrombotic events, anticoagulation with heparin along with eculizumab should be started immediately and sometimes even local or systemic thrombolytic therapy.

After a thromboembolic event, patients should be anticoagulated indefinitely and treatment with eculizumab initiated. In a retrospective study, the risk for thromboembolic events was related to the PNH clone size. Patients with a PNH clone size >50% (as measured by GPI-deficient granulocytes) should preferentially be treated with eculizumab; however, those patients not indicated for eculizumab and normal platelets should be prophylactically anticoagulated. Furthermore, prophylactic anticoagulation preferentially with low molecular heparin is also recommended in high-risk situations for thrombosis like surgery or pregnancy, despite eculizumab treatment. So far, discontinuation of primary or even more secondary prophylaxis remains controversial, and more long-term results and further studies are needed. Individual decisions on primary prophylaxis should be based on symptoms, clone size, other thrombophilic risk factors, platelet count, medication including eculizumab, age, activity level, compliance, and patient preferences.

Stem cell transplantation
PNH in the setting of AA treatment should be directed to the underlying bone marrow failure. If the criteria for severe AA are met, patients should go for allogeneic SCT or immunosuppressive therapy depending on the age of patient and availability of suitable HLA-matched donor. Furthermore, and according to the IPig criteria, SCT should also be considered in the situation of major complications of PNH (e.g., refractory, transfusion-dependent hemolytic anemia or recurrent life-threatening thromboembolic disease).

Conclusions
The advances in the understanding of the pathophysiology of PNH over the last decades have led to a highly effective and targeted therapy with eculizumab. With
TREATMENT OF PNH

its approval, a targeted and disease-modifying treatment option is available that is well tolerated and reduces hemolysis, fatigue, anemia, transfusion requirements, renal impairment, pulmonary hypertension, and the risk for thromboembolic events and improves anemia and the quality of life. Eculizumab has therefore become the gold standard treatment for hemolytic PNH patients, even leading to a major improvement in survival, as demonstrated previously by Kelly et al.40

Moreover, eculizumab has initiated an expanding new era of complement modification as a therapeutic strategy and will offer new options for the treatment of other complement-mediated diseases such as atypical hemolytic uremic syndrome,41,42 antibody-mediated transplant rejection,43 and hemolytic cold agglutinin disease.44

Acknowledgements This review is dedicated to Prof. Dr. Günter Brittinger on the occasion of his 80th birthday.

Conflicts of interest A.R. received lecture fees from Alexion Pharmaceuticals and served on advisory boards for Alexion Pharmaceuticals.

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TREATMENT OF PNH

Blood supply in the Kingdom of Saudi Arabia—self-sufficiency and safety considerations

Abel Galil M. Abdel Gader, Farga H. Alqahtani, Abdulmajeed A. Albanayan

The transfusion of blood and its derivatives is a vital supporting service to clinical medicine. However, over the years, 2 considerations have been of major concern to both health planners as well as professionals in charge of blood banks, namely, self-sufficiency and safety.

In the Kingdom of Saudi Arabia, the blood transfusion service is predominantly a hospital-based blood banking system. Despite the shortcomings of this system, self-sufficiency has been attained with respect to fresh cellular components (packed red blood cells and platelet concentrates) and plasma derivatives (fresh frozen plasma and cryoprecipitate). However, since the requirement for hemotherapy is phasic in nature and variable in quantity, hospital blood banks are exposed to frequent shortages in the supply of single components when heavy demands of that component arise.

As to the second issue of safety, specifically reducing the risk of infection with transfusion-transmitted pathogens, it is addressed satisfactorily by undertaking newly emerging screening assays, including nucleic acid testing for hepatitis B and C and human immunodeficiency viruses. The continuous expansion in the number and sophistication of assay techniques designed to detect an ever-increasing number of pathogens leaves a lot to be desired. Malaria, for which there is no specific and sensitive screening test, remains a daunting challenge.

Additionally, viral inactivation of the frequently consumed fresh frozen plasma as well as universal leucodepletion is yet to be implemented in all blood banks. Current efforts led by the Ministry of Health towards establishing a unified national blood transfusion service, based on non-remunerated voluntary donors, is a dream that should not take long to come true and will no doubt be the ultimate answer for self-sufficiency and safety.

Since the discovery of the ABO blood group system by Landsteiner and his co-workers in the early years of the 20th century, blood transfusion has developed into a multifaceted medical discipline based on scientific knowledge and highly developed technology. This has been the result of the extensive interaction between blood transfusion and many basic sciences, including immunology, chemistry, biochemistry, physiology, genetics as well as engineering and computer science and technology. Throughout and up to this date, blood transfusion has kept its place as a vital supporting service to clinical medicine. As more and more advances are gained by clinical medicine, blood transfusion has kept pace and has also scored uncountable developmental steps. For example, the constant advances in cardiac and transplant surgery, bone marrow transplantation, and chemotherapy of malignancies, especially hematological malignancies, would not have been possible without the support of blood transfusion. Recent developments have led to better exploitation of the limited available blood resources, increased safety, new therapeutic options, and even alternatives to blood transfusion, especially oxygen carriers (so-called blood substitutes).

However, it is the danger of transmitting infectious diseases, particularly human immunodeficiency virus (HIV), that has provoked intense medical as
well as public concern on the safety of transfusion. Although it is understandable that patients and their treating physicians should worry about blood transfusion therapy, such worries have reached blood donors who request reassurance on the safety of even blood donation. These perspectives have prompted major changes in the field of blood transfusion in recent years. This is particularly evident in developing countries where blood transfusion service (BTS) is nowadays being managed on market basis, governed by good manufacturing practices, and the balance has shifted from blood transfusion being a service freely available to needy patients to a form of drug therapy with its consequent accountability and liability. This whole issue has been compounded further by financial constraints that ended in the management of blood transfusion falling gradually into the hands of administrators from outside the blood transfusion specialty.

While these developments are taking place in the industrial countries, with well-developed and very sophisticated national (or Red Cross) BTSs, the developing countries have lagged behind and are struggling to sustain the minimum standards of BTS. They have also been isolated to a great extent from sharing the many advantages and developments prevailing in the industrial countries.

Where does BTS in the Kingdom of Saudi Arabia (KSA) stand? This review attempts to explore the current status of BTS in the KSA, addressing 2 major transfusion issues, namely, self-sufficiency and safety.

Where Does BTS in the KSA Stand?
An important prerequisite to the answer to this question is the definition of the aims of the BTS that can be summarized as follows:

i. Provision of adequate, safe, and effective blood products
ii. Care of the donor, donation, and recipients
iii. The optimal use of the available donor blood.

These aims remained unchanged for decades. However, the extent to which they are fulfilled has been dominated by growing concerns about self-sufficiency and safety in the face of dwindling financial support, a constantly changing BTS management structure (whether it being independent blood banking system or national service or part of the hospital laboratory service) as well as more and more intrusion by the legal system.

Blood Transfusion in Saudi Arabia:
The main provider of health services in the KSA is the Ministry of Health. There are also University, Military, National Guard, Security Forces Hospitals, as well as Private Hospitals. These different providers are totally independent of each other. Accordingly, the BTS, which is mainly a hospital-based blood banking system, is spread in the form of hospital blood banks all over the KSA, and every hospital (small or large) has its own blood bank to cover its needs of blood and its derivatives. Therefore, blood banks are everywhere because hospitals are everywhere. In the large provincial capital cities (Riyadh, Jeddah, and Dammam), BTS assumes regional character since the Central Banks in the major cities, which are part of the major Ministry of Health Central Laboratories, supply blood products not only to the main hospitals within the city boundaries but also to smaller provincial hospitals.

As expected the responsibilities of these widespread Hospital Blood Banks include:

i. The collection of blood from donors
ii. Testing the blood for infective agents
iii. Processing of donated blood units and the preparation of packed red blood cells (RBCs), fresh frozen plasma (FFP), and cryoprecipitate (in large blood banks)
iv. Storage and issue of blood products.

The main sources of blood donations are:

i. Relatives of patients admitted to hospitals and whose care requires hemotherapy, particularly elective surgery. Hospital Blood Banks are currently following the rule: “No Blood - No Operation.” The major drawback of this system of forced donations is that the concern and response is short-lived.
ii. Voluntary donor recruitment: This takes the form of either the blood banks sending their collection teams to the various government departments, educational institutes particularly universities, higher colleges, security and military forces, factories and large commercial businesses, etc. or the increasing number of voluntary donors who are taking the habit of regular donations
iii. Autologous donation is also practiced but on a very limited scale.

As mentioned earlier, in most blood banks, the blood products that are prepared from whole blood
donations are both cellular components (packed RBC and platelet concentrate) and plasma derivatives (FFP and cryoprecipitate).

Self-sufficiency?

Definition:
Self-sufficiency refers to the ability of the BTS to cover the patient’s requirement for blood derivatives, including plasma fractions. Accordingly, the KSA has not yet attained self-sufficiency with respect to certain blood derivatives, especially the following plasma fractions, which are all imported from commercial sources: clotting factor concentrates (especially FVIII), albumin, PPF, immunoglobulins, and vaccines.

The important question then arises: Is it possible for the KSA to attain self-sufficiency in these plasma derivatives? To answer this question let us take the example of clotting factor VIII concentrate, the mainstream of hemophilia replacement therapy, as an example.

Although the exact number of hemophiliacs in the KSA has not been ascertained, if we take the international estimate of 50 hemophiliacs per million, then the estimated number of hemophiliacs in KSA is expected to be 900 patients. If we assume the annual requirement for each patient to be 200,000 IU of factor VIII concentrate, the total requirement will be 18 million IU per year. The estimated need for donated plasma to prepare this quantity of factor VIII concentrate will be 450,000 L/year. This means that the Ministry of Health blood banks (alone) should double its current number of collected blood donations to meet the plasma requirements for clotting factor VIII preparation by some manufacturing facility whether within or outside the KSA.

Such a large increase in the number of donors raises a more relevant question: Have we tested the extent of the donor potential in Saudi Arabia?

The Gulf War Experience:
The answer to this question is a certain yes. During the Gulf War (1990), the blood banks in the major hospitals in Riyadh, in anticipation of the excessive war casualties, waged a very active donor campaign well before the actual fighting commenced in February 1990. As expected, the response to these donor recruitment campaigns has been overwhelming. For example, the mid-week packed RBC inventory for King Khalid University Hospital (KKUH) alone and all major Riyadh hospitals’ blood banks are shown in Figures 1 to 3.

It is clear that the inventory increased from 3 times in the Central (Ministry of Health) Blood Bank to 7 times for the KKUH Blood Bank, and the total inventory of packed RBCs in Riyadh increased about 4 times. Thus, the potential for increasing the current donor blood collection is enormous. It may be argued that the donor call in the war situation is expected and could be short lived; nonetheless, it was a test of donor potential.
King Saud University (KSU) Students Donor Drive—The Peace Experience

This donor drive, which is organized by the Deanship of Student Affairs, KSU, started in 1394 (1973) with 13 donors in its first year to reach 4500 donors in the academic session of 1407–1408 (1986–1987) (Figure 4). The donors are mainly university students. The incentives given to donors, include wristwatch, briefcase, and headdress (ghutra). A “University Blood donation Trophy” is awarded annually to the college that donates the maximum number of units. Each year the university rector is the first to donate, and his inauguration of the campaign is given wide publicity within and outside the university.

The KSU donor drive is a model, which is gradually being followed by other educational, civil, and military institutes as well as government departments. It forms an excellent basis for future national voluntary blood donation. Two remarks need to be added on the enormous potential of the KSU donor drive: Firstly, most donors donate only once/year, i.e., the total number could easily be doubled if the current donors give their donations at least twice/year. Secondly, no target figure for the total number of donations was set. Indeed, the current number of donors could easily be increased 4 to 5 times, if use could be made of the collected blood and its derivatives. As seen in Figure 4, the total number of donors dropped markedly in recent years, and this is due to the fact that donor collection teams started to get calls from numerous educational institutes, government departments, and private companies to come over to collect blood from their employees. A closer look at Figure 4 indicates that the potential at KSU alone could cover the annual needs of KKUH for blood and its derivatives without resorting to the forced recruitment of donors from relatives of patients, i.e., the “No Blood - No Operation” rule. The number of enrolled students at KSU which about 30,000 10 years ago, now stands at around 120,000 students. If we add the number of staff, both academic and nonacademic, and their families the total potential donor population at KSU could be well above 200,000.

What is needed now is the exploitation of this potential for expanding the donor pool, and the dependence in totally voluntary non-remunerated donor system. This entails proper planning backed by appropriate legislation to integrate the current donor recruitment activity, targeting a specific total donor input to cover the current needs for blood and its derivatives and also to help plan for the future plasma fractionation facility. This should eventually transpire into reliance on voluntary blood donations. Voluntary donor recruitment is currently very successful and is expanding as more and more donors are building the tradition of regular blood donation. Ultimately, when service is well integrated and organized, full scale voluntary service could be established.

This discussion will not be complete without referring to a widely referenced document relating the establishment of BTS in developing countries. The World Health Assembly in 1975 passed the following resolution (Resolution 25.72):

“…member states to promote the development of National Blood Services based on voluntary non-remunerated donations of blood…”

However, various countries are facing some of these problems, and it is taking them too long to establish a national BTS. Nonetheless, this resolution has set the stage for developing countries to take the necessary steps to reach this aim. Success has already been scored in Hong Kong, Zimbabwe, South Africa, Iran, where the following main features of a National Blood Transfusion Service are already established:

* Blood is given free
* BTS is organized without profit on a national scale
* Blood and its derivatives are made available to patients at any time in the quantities needed.

Blood Safety

The general approaches to secure safety of transfusion of blood and its derivatives can be summarized in the following:

* Education, questioning, and selection of donors
* Routine serological screening of all donations for a range of microbial infections
* Enhancements in automation of testing
* Computerization
* Collation and monitoring of test performance, on a national basis
* Test kit evaluations for suitability
* Viral inactivation of fractionated plasma products
* Viral inactivation of full range of blood components
* Leucodepletion (impact on bacteria, CMV, EBV, HHV, HTLV, and vCJD)
* Detection of microbial nucleic acid (in mini-pools or in individual samples).

Despite marked advances in medical sciences and the marked improvement in specificity and sensitivity of the pathogen detection techniques, blood transfusion is still fraught with risks, and zero-risk blood transfusion does not exist. The potential risks to blood transfusion include:

* Risks of infection: This remains the most significant risk of transfusion of blood derivatives. The infective microbial agents could be bacteria causing septic toxic reactions, protozoa (syphilis and malaria), and viruses (HIV, hepatitis A, B, C, D, and E, retrovirus HTLV, and CMV). However, for practical purposes, screening is performed on a limited number of these infective agents (Table 1).11-13
* Risks related to technique and physics of transfusion (cooling, air embolism, microaggregation, and circulatory overload)
* Biochemical-metabolic risks (citrate intoxication, coagulation deficiencies).

General approaches to secure safety have been identified, and different blood banks follow these approaches depending on the available financial resources, alignment, and/or membership of international blood banking accreditation bodies, e.g., American Association of Blood Banks (AABB), and above all conforming to the guidelines of the Ministry of Health. These guidelines oblige all blood banks in the KSA to perform all the tests in Table 1. Nucleic acid testing (NAT) was recently introduced, and all blood banks are obliged to perform NAT for hepatitis viruses B and C as well HIV for all the collected donor blood and its derivatives. We may add here that NAT, which allowed the direct detection of HIV and HCV and HBV, has also helped in elimination of the window period before seroconversion for viral antibody.13,14

Despite wide disagreements on the sensitivity and specificity of the current immunological assays for malaria parasite,15-18 the Ministry of Health obliges blood banks to undertake a suitable test on donated blood.

Figure 5 shows the prevalence of HBV (HBSAg) and hepatitis C antibody in blood donors at King Khalid University Hospital, Riyadh, in the period from 1413 to 1428.
Other than the battery of screening tests (including the ascertainment of quality assurance of the proficiency of these test), viral inactivation of plasma products, including FFP, and leucodepletion and its effect in removing the risk of transmitting CMV and HTLV viruses\textsuperscript{22-27} are waiting to be introduced in all blood banks in the KSA. Also, as the sensitivity of the screening tests for various pathogens is getting to their limits, further refinements in these tests will yield little further reduction in the risk of viral infectivity, leaving more emphasis to be focused on health education and selection of donors particularly the donor questionnaire.\textsuperscript{22-27}

This approach should also focus on the identification and keeping of the so-called “safe” donors.

**Safe Donors:**
By definition, safe blood donors are those whose regular donations were negative on screening tests and who reported no behavior risk factors on post-donation survey.\textsuperscript{28} Further specific characteristics of a “safe” donor have been outlined recently;\textsuperscript{28-29} these characteristics include repeat donors, women, donors aged \( \geq 45 \) years, and donors with more education.

Lastly, we have to bear in mind that since blood is a biologic product, it is unlikely that the risk for transfusion-transmitted infection will ever be reduced to zero. A recent review has identified 2 elements involved in the provision of safe (infection-free) blood products:\textsuperscript{29}

- **Production Process:**
  This concentrates on the donor area and deals with donor education, selection, testing, and exclusion. The concept of “safe” donor already dealt with above summarizes the production process satisfactorily.

- **Clinical Supply Process:**
  This involves the actual transfusion process from the moment a decision is made that a blood product should be transfused to a patient and then getting the right blood, to the right person, at the right place, at the right time. Examples of failures in the clinical process include patient receiving the wrong blood products, transfusing inappropriate doses of blood products, blood arriving late in an emergency, or errors and adverse reactions concealed.

Recently, the seriousness of administrative errors was reported; earlier by McClelland & Phillips\textsuperscript{30} and more recently by others\textsuperscript{21-33} who found the frequency of deaths due to patients receiving wrong blood to be 30-fold higher than current estimates of transfusion-transmitted HIV infection. Also, in a 5-year period, 22 young women with major obstetric bleeding died due (at least in part) to delays in administering RBC transfusion (Department of Health, UK, 1994).\textsuperscript{34} Lastly, it must be noted that most decisions of emergency transfusion are taken by busy clinicians who are young, inexperienced, fatigued, and poorly supported by senior colleagues.

The experience of the last 3 decades confirmed further the following steps to be most effective in reducing the risk of transmitting blood-borne infections:\textsuperscript{21,22,26-29,33}

- Elimination of paid donors
- Refinements of methods of recruiting donors (specificity of health history)
- Implementation of highly sensitive continuously refined blood screening tests, including molecular testing for viral agents that eliminate the window period for viral infections
- Viral inactivation of plasma for transfusion. It will not be too long before viral inactivation of cellular blood components will be devised.

**Where Do We Go From Here?**
The experience of well-established BTS, particularly, in industrialized countries can allow us to find general answers to this question and that may activate direct efforts and generate practical steps for the future development of BTS in Saudi Arabia. First and foremost, BTS must be given a separate and independent identity (including administration) and not to be taken as part of a “laboratory service.” Given this identity, then BTS can move forward and fulfill the following goals:

1. Development and implementation of (evidence-based) standards for:
   - Blood transfusion
   - Clinical guidelines
2. Quality Assurance
3. Objective indicators of achieved safety and efficacy should be developed, validated, and used
4. Clinicians, patients, and donors should be assured and made aware of these steps
5. Financial Support: All the above steps cannot succeed without generous financial support.

There is evidence that money is available should health planners take the right decision for the future development of BTS in Saudi Arabia and guarantee the fulfillment of self-sufficiency and safety of blood and its derivatives.

Dr. A F Britten, Former Head, Blood Program, International Federation of the Red Cross and Red
Crescent Societies (International Blood Transfusion, Size of the problem). Countries have summarized the following problems of blood transfusion in the developing world:

* Cultural/Religious attitudes discourage blood donation
* Public not educated to the need for blood donation
* Excess plasma but no facilities for plasma fractionation
* Money not available for major equipment
* Transportation system inadequate for blood deliveries
* Power failures cause equipment breakdown
* Equipment maintenance not available
* Person responsible for blood transfusion[^1] is not given the authority/power to carry out responsibility
* Blood donations inadequate to meet need

* Government does not recognize the importance of blood transfusion
* No building facility suitable for a blood center
* No technical expertise
* Competition for voluntary donors
* No quality control exist
* Military, private, social security, Red Cross, and university blood banks do not cooperate.

It is clear that none of these problems exist in the KSA. Indeed, if one is to scale the international standing of current services offered by the BTS in KSA, it lies comfortably ahead of most, if not all, developing countries. Most blood banks either are accredited by the AABB or have started the accrediting procedure. Steps towards unifying the BTS have already commenced under the auspices of the Ministry of Health. So, the establishment of a Saudi National Blood Transfusion Service is a dream that should not take long to be true.
References

Thromboembolic complications occur due to major surgery or several clinical conditions such as hypercoagulable state. The risk of venous thrombosis due to acquired hypercoagulability is directly related to age and elder individuals are more susceptible to this condition. The probability of thrombosis increases by 100 fold from the age of 40 to 75.1 Multiple acquired factors such as obesity,2 hospitalization,3 trauma,4 surgery,5 and immobility6 can modify the onset age and/or the clinical presentation of thrombosis directly or in concert with inherited elements.

Inherited thrombophilia due to natural anticoagulant deficiencies or gain of functions can modify the onset age and/or the clinical complications of thrombosis.

Deficiency of natural coagulation inhibitors such as protein C (PC), protein S (PS), and antithrombin III (AT III) is reported to occur in less than 1%6-8 of the general population. However, the second group of genetic factors (i.e., gain of function), including factor V Leiden (FVL) and its HR2 haplotype,9 the prothrombin G20210A mutation (PT 20210A),10 and elevation of procoagulant factors such as factor VIII, von Willebrand factor, and factors V, VII, IX and XI11 are more prevalent than natural anticoagulants.

Depending on the nature of inherited thrombophilic defect(s), the spectrum of clinical complica-
original article

Inherited thromboembolism in Iran

Journal of Applied Hematology 2011

Inherited thrombophilic factor deficiencies vary from mild to severe venous thrombosis. The frequency of each inherited risk factor varies among different ethnic groups, and a high frequency of consanguinity increases the homozygosity of each defect separately or in combination with the others. Only a few studies have been performed on the frequency of genetic defects in the Iranian population, and the impacts of genetic defects on the development of deep vein thrombosis (DVT) in individuals in south Iran is not well defined. The aim of this study is to determine the frequency and influence of the following factors on DVT in individuals in south Iran: inherited PC and PS mutations, inherited AT III deficiency, and the presence of PT 20210A and FVL mutations.

Patients and Methods

During a period spanning 1 year, 135 patients with venous thrombosis and 1200 randomly selected healthy blood donors were enrolled in the study. The local ethics committee approved the study, and informed consent was obtained from all participants.

Laboratory tests

Blood samples were collected before the patients started taking any medications and at 10 days after holding anticoagulant therapy with Na-citrate (109 mmol/L) and platelet-poor plasma was initiated; the samples were frozen and stored at -70ºC until testing. The activities of PC, PS, and AT III were measured in accordance with the manufacturer's instructions (Albion, France). The resistance to proteolytic degradation by activated PC (APC-R) was measured using a kit (Instrumentation Laboratory Company), and the results were expressed in terms of normalized ratios.

Molecular analysis

For PT 20210A and FVL mutation analysis, genomic DNA was extracted from white blood cells and amplified using polymerase chain reaction (PCR). Mutation analysis for both genes was performed using restriction fragment length polymorphism (RFLP) and high-resolution melting (HRM) analysis (Rotor-gene 6000), as described in a previous study.

Statistical Analysis

The χ² test was used for statistical analysis. The prevalence of odds ratios (ORs) was considered as the prevalence of existing disease, and 95% confidence intervals (CIs) were calculated using normal approximation; P values less than 0.05 were considered significant. Data were statistically analyzed using the student t test and Mann-Whitney U test, and a P value of <0.05 was considered significant.

Results

All the target genes were autosomal, and no gender priority was observed. From the 1200 healthy individuals, 4 individuals with PC deficiency, 3 with PS deficiency, and 1 with AT III deficiency were identified. The mean estimated frequencies of PC, PS, and AT III deficiencies with 95% CI were 0.33% (range, 0.30–0.36%), 0.25% (range, 0.23–0.27%), and 0.08% (range, 0.06–0.10%), respectively. Mutation analysis of the genomic DNA samples revealed 4 heterozygotes and 1 homozygote of PT 20210A; 46 individuals were FVL carriers, and 1 case of homozygosity was identified (Table 1).

The patient group included 64 males and 71 females; their ages ranged from 24 to 65 years. All the patients with lower limb DVT underwent Doppler color sonography or venography to confirm the diagnosis of DVT.

The normal range was set at 70–130% for PC activ-

<table>
<thead>
<tr>
<th>Protein C</th>
<th>Protein S</th>
<th>Antithrombin</th>
<th>PT 20210A Allele*</th>
<th>1691 A (FVL) Allele*</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (%) 95% CI</td>
<td>n (%) 95% CI</td>
<td>n (%) 95% CI</td>
<td>n (%) 95% CI</td>
<td>n (%) 95% CI</td>
</tr>
<tr>
<td>DVT</td>
<td>7 (5.2)</td>
<td>5 (3.7)</td>
<td>6 (4.4)</td>
<td>49 (18.1)</td>
</tr>
<tr>
<td>Control</td>
<td>4 (0.33)</td>
<td>3 (0.25)</td>
<td>1 (0.08)</td>
<td>42 (1.75)</td>
</tr>
</tbody>
</table>

*Alleles per chromatid
Discussion

DVT is a multifactorial disease and is influenced by acquired factors, genetic factors, or both. The genetic and environment act synergistically in the development of DVT. The proportion of the genetic risk factors in certain populations, and consequently, the priority of each risk factor and the possibility of co-inheritance of these factors are variable. Although the incidence of thrombosis before the age of 40 years is approximately 1 in 10,000 individuals per year,1 thrombosis can occur at a young age because of certain inherent parameters.

The gene encoding PC is located on chromosome 2q13-q14 and has 262 reported mutations.16 The gene consists of 9 exons and encompasses 11 kb of DNA.7 Type I and II deficiencies can be diagnosed using functional analysis, but an antigen assay or DNA analysis is required for differential diagnosis.

The gene encoding PS has 15 exons, spans 80 kb, and is located on chromosome 3q11; it acts as a cofactor for activated PC.17 In 1994, Reitsma first reported mutations in the PROS1 gene;18 to date, approximately 200 mutations have been reported for this gene.16 All the types of PS gene mutations result in low protein activity; only type I and III deficiencies are characterized by low antigen-free PS levels. As a rule, 60% of inactive PS is bound to C4b; its level decreases in type I deficiency but is normal in type II and III deficiencies. We are now performing a DNA-based study on PC and PS deficiencies.

The interpretation of the results of the functional assay for the diagnosis of AT III deficiency is slightly complex in the case of type II deficiency and depends on whether the defect at the active site or heparin binding site have different patterns, but in type I deficiency, the results of both assays (antigenic and functional) are always low.

APC-R is characterized by prolonged clotting after adding activated PC. Diagnosis based on conventional coagulation-based methods for APC resistance is not highly specific with respect to genetic defects. In contrast, DNA-based assays are highly specific and are not affected by pregnancy, contraceptive use, anticoagulant therapy, or inhibitors.19 Approximately 7% of individuals who have abnormal APC-R do not have defects in any of the abovementioned factors.

The carrier frequency of FVL (R506Q), a missense mutation, is 1 in 25, and approximately 1 in 1000 homozygous individuals in the south Iranian population are at risk of developing thrombosis in the. In our study, the frequency of the FVL allele in the patient group was significantly higher than that in the controls subjects (0.196 vs 0.021, \( P < 0.001 \)).

The prevalence of the FVL allele among Europeans

Table 2. Frequencies of factor V Leiden mutation and prothrombin (G20210A) genotypes in DVT patients from Shiraz University hospitals and in healthy controls (asymptomatic).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Observed Genotype in Patients (%)</th>
<th>Expected Genotype in the Population (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVL 1691 A/A+ PT20210 A/G*</td>
<td>5.2</td>
<td>0.1376</td>
</tr>
<tr>
<td>FVL 1691 A/A+ PT20210 A/A**</td>
<td>2.2</td>
<td>0.0012</td>
</tr>
<tr>
<td>FVL 1691 A/A***</td>
<td>6.6</td>
<td>0.0400</td>
</tr>
<tr>
<td>FVL 1691 A/G</td>
<td>10.6</td>
<td>3.9200</td>
</tr>
<tr>
<td>PT20210 A/A</td>
<td>9.6</td>
<td>0.0300</td>
</tr>
<tr>
<td>PT20210 A/G</td>
<td>6.8</td>
<td>3.4400</td>
</tr>
</tbody>
</table>

*Compound homozygote FVL + Heterozygote PT, **Compound homozygote FVL + Homozygote PT, ***Homozygote FVL.
is also high: Greece, 0.070; United Kingdom, 0.044; Germany, 0.033; and France, 0.017. In our study, the frequency of this mutation was 0.021 and was significant; the thrombotic risk in the carriers was high (OR=11.9; 95% CI, 5.6–25.5). These results were in contrast to those of several studies that showed a low frequency of this mutation in people from Asia, Africa, and the Far East, and were in agreement with those of another study where a frequency of 0.014 was reported for individuals from west Iran.

The prothrombin G20210A mutation is also common among Europeans, but the data on its frequency in Asians and Africans are controversial. Currently available data suggest that the frequency of the PT 20210A mutation increases from the Northern Hemisphere to the Southern Hemisphere. The frequency of this allele is even higher in Saudi Arabia than in South of Iran. In our study, the PT 20210A mutation was detected in 23.7% of patients with DVT and in 3.1% of the control group. These values are higher than those reported for Sweden (7.1%, 1.8%) and the United Kingdom (5.5%, 1.2%), and the difference between the groups was significant (P>.05). This might be related to the relatively high frequency of this allele in the population studied.

The overall risk of recurrent DVT in patients with compound heterozygous FVL and the PT 20210A mutation is 2.7 and 2.6 times higher, respectively, than that in patients who do not have these mutations or have FVL alone. In the current study, 10 of 56 patients had coinheritate of FVL and PT 20210A (Table 1).

An HR2 haplotype of factor V has been reported that can contribute by itself to a mild APC resistance phenotype and can synergistically interact with the FVL mutation or PT 20210A to produce a severe APC resistance phenotype. This haplotype is associated with more than 12 polymorphisms of the factor V gene, which are collectively known as HR2. This haplotype is associated with an increased risk of developing venous thrombosis as well as arterial thrombosis. In our study, the frequency of this haplotype in the patients with DVT was significantly higher than that in the control group (data not included).

Considering that the incidence of PC, PS, and AT deficiencies in the general population is low; the number of controls used in this study is not sufficient for estimating the frequency of these deficiencies in the south Iranian population.

A few studies have compared the combined hereditary risk factors according to the clinical manifestations of venous thromboembolism.

In conclusion, we found that the frequencies of the FVL and PT 20210A mutations in the patient group were greater than those in the control group. Coexistence of hereditary thrombophilic risk factors lowers the onset age, increases clinical severity, and causes recurrent DVT. Family-based genetic counseling and prophylaxis therapy can reduce the adverse effect of thrombosis. Identifying genetically predisposed individuals would aid in achieving this goal.
INHERITED THROMBOEMBOLISM IN IRAN

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Endocrinopathies in Children and Adolescents with β-Thalassemia Major

Abdulmoein Al-Agha, Shadi A. Shabakah, Ali Ocheltree, Daniah El-fateh M. Abdullatif, Soad K. Al Jaouni

OBJECTIVES: β-Thalassemia major (β-TM), a prevalent medical condition, is associated with multiple endocrinopathies. We aimed to evaluate the prevalence of endocrinopathies between 2006-2010 in children and adolescents with β-TM and were 2-18 years old.

PATIENTS AND METHODS: This retrospective study involved children and adolescents with β-TM (n=143, 62 females, 57.81% were pubertal, and 81 males, 56.96% were pubertal) presenting at the pediatric endocrine clinic at King Abdul-Aziz University Hospital. The mean and standard deviation (SD) for age were 10.96 and 4.4. A comprehensive review of patient serum analysis, and medical records were done.

RESULTS: Vitamin D (Vit. D) deficiency was the commonest (56%) endocrinopathy in both children and adolescents with β-TM, followed by pubertal delay (29.37%) and hypothyroidism (21%); 7.6% of the patients had no endocrinopathies, and 45.5% had 3 or more endocrinopathies. Growth hormone deficiency was observed in 12.58% of the patients. The overall mean and SD serum ferritin levels were 3400.86 and 3067.43 ng/mL, respectively. Iron overload worsened as the children grew older; the mean and SD serum ferritin levels were 2893 and 1919 ng/mL, respectively, for pre-adolescents and 4299 and 4276 ng/mL, respectively, for adolescents (P=0.0368 [S]).

CONCLUSION: Children and adolescents with β-TM are at risk of multiple endocrinopathies. Vit. D deficiency, delayed puberty, short stature, and hypothyroidism are the prevalent complications of iron overload. We recommend the promotion of early screening programs for iron overload to prevent endocrinopathies among children and adolescents with β-TM and prophylactic Vit. D supplements.

KEYWORDS: β-Thalassemia major, Endocrinopathy, Vitamin D deficiency, Iron overload, Children.

Children who have β-thalassemia major (β-TM) and undergo multiple transfusions may develop severe endocrine complications because of iron overload.1 The anterior pituitary gland is particularly sensitive to iron overload, which disrupts hormonal secretion resulting in delayed puberty; short stature; adrenal insufficiency; and acquired, clinical, or subclinical hypothyroidism.2,3 Such endocrinopathies hinder growth, ultimately decreasing adult height.4 Delayed or absent puberty are common complications affecting many adolescents with β-TM.4 Glucose intolerance (GI) in adolescence and diabetes mellitus (DM) later in life are also frequent complications and mainly develop because of iron overload, chronic liver disease, and genetic predisposition.5 Early recognition of endocrinopathies and their prevention by performing early and regular chelation therapy is imperative for improving the quality of life and psychological outcomes of these patients.5 The purpose of this study was to determine the prevalence of endocrinopathies in children and adolescents who had β-TM and visited the pediatric endocrine clinic.
at King Abdul-Aziz University (KAAU) Hospital between 2006-2010.

**Patients and Methods**

**Study design and site**

This is a retrospective cross-sectional study involving children who had β-TM and visited the pediatrics endocrine clinic at KAAU Hospital between 2006-2010. We reviewed the data on endocrinopathies in the clinical files of all children and adolescents who had β-TM. All the laboratory test data were obtained from KAAU Hospital laboratory database.

**Study Subjects**

The study population comprised 143 pediatric patients who were between 2-18 years of age (female patients, 62; male patients, 81). Local patients of multiple nationalities were enrolled in this study (Saudi patients, 24.75%; non Saudi patients 75.25%). The mean, SD and median for the patient’s age were 10.96, ±4.4 and 11 years, respectively. Mean and SD for female’s age were 11.35 and ±4.75 years, respectively, 37 (57.81%) of females were pubertal. Mean and SD for male’s age were 11.44 and ±4.06 years, respectively, 45 (56.96%) of males were pubertal.

The inclusion criteria were as follows: age between 2-18 years; pretransfusion hemoglobin level >9 g/dL; high ferritin levels >1000 ng/mL. In addition, all the patients enrolled in this study were diagnosed with β-TM both clinically and via laboratory methods (hemoglobin electrophoresis) and required regular monthly blood transfusions before 2 years of age.

**Diagnosis of Endocrinopathies:**

The following parameters were reviewed: serum levels of calcium, phosphorous, alkaline phosphatase enzyme, parathyroid hormone, 25-hydroxyl Vit. D, fasting glucose, thyroid-stimulating hormone (TSH); free thyroxin, (FT4), adrenocorticotrophic hormone (ACTH), morning cortisol, serum level of insulin like growth factor -1 (IGF-1), growth hormone (GH). Stimulation test in those with short stature, luteinizing hormone (LH), follicular-stimulating hormone (FSH), and estradiol in girls above the age of 10 years and testosterone in boys above the age of 12 years were also reviewed. The normal reference ranges for the laboratory variables reviewed in this study are given in Table 2. We compared the serum ferritin levels of the patients with respect to age and the endocrinopathies (normal vs. affected) that they had developed. We defined the onset of normal puberty as the development of thelarche by the age of 8 years or older in girls, and testicular enlargement, measured using the Prader orchidometer, by the age of 9 years or older in boys. Delayed puberty in girls was defined as the absence of breast development by the age of 13 years and/or absence of pubic hair by the age of 14 years, and primary amenorrhea was defined as the absence of menarche by the age of 16 years or a time gap of greater than 5 years between thelarche and menarche. In boys, puberty was defined as delayed if testicular enlargement, as measured using a Prader orchidometer, was less than 4 mL by the age of 14 years and/or pubic hair did not develop by the age of 15 years, or complete genital enlargement was delayed for more than 5 years. We defined short stature as an SD score of height less than -2 below the mean for age, gender, and ethnicity. GH deficiency was defined by a maximum peak of GH values less than 10 ng/mL by 2 pharmacological provocative agents (clonidine and glucagon agents were used as stimulants). The existence of primary hypothyroidism was established via evidence such as greater than normal levels of thyroid stimulating hormone and low or normal free thyroxin levels. Hypocalcemia in our study population was defined as corrected total serum calcium values of less than 2.1 mmoL/L with respect to phosphate levels that were adjusted according to age. Vit. D deficiency was defined as levels less than 75 nmoL/L. We defined adolescents as children in the 13-18-year age group and preadolescents as children younger than 13 years.

**Statistical analysis**

The clinical and laboratory information was collected on a datasheet from KAAU Hospital Phoenix database and was digitized on an IBM CPU. The mean, SD, and median values for age and serum ferritin levels, and the frequencies of endocrinopathies were calculated. Microsoft Excel 2010 software was used for the data analysis and table formulation and presentation of the presented study findings. Student t-test was performed for qualitative data analysis. The level of significance was expressed in terms of P-values: $P>0.05$=non significant (NS), $P<0.05$=significant (S), and $P<0.001$ = highly significant (HS).

**Results**

Iron overload worsened as the patients grew older. The mean, SD, and median values for serum ferritin for preadolescents were 2893, 1919, and 2387 ng/ml, respectively, and the mean, SD, and median
values for serum ferritin for adolescents were 4299, 4276, and 2543 ng/mL, respectively (P=0.0368 [S]). From the study population, 7.6% had no endocrinopathies, 22.4% had a single endocrinopathy, 24.5% had 2 endocrinopathies and 45.5% had 3 or more endocrinopathies. Short stature was seen in 16.78% of patients with an SD score of height less than -2 (Table 1). There were no cases of primary hypogonadism or cases of secondary hypothyroidism. The mean and SD for serum calcium levels were 2.18 ±0.145 mmol/L. The serum calcium level was lower than normal in 45.4% of the patients and 51.05% had elevated alkaline phosphatase levels. The mean and SD for patient overall serum ferritin levels were 3400.86 and 3067.43 ng/mL, respectively.

Delayed bone age was seen in 14.69% of the patients. The serum ferritin mean, SD, and median serum ferritin values for children with normal bone age were 2752.6, 2053, and 1895 ng/mL, respectively, and for children with delayed bone age were 5333, 3430, and 3357 ng/mL, respectively (P=0.033 [S]). Primary hypothyroidism was observed in 21% of patients (mean±SD age, 10.81±4.4 years). The mean, SD, and median serum ferritin values for children with normal TSH levels were 3376, 3114, and 2503 ng/mL, respectively, and for children with low TSH levels were 3676, 3184, and 2543 ng/mL, respectively (P=0.623 [NS]). The mean, SD, and median serum ferritin values for patients with low Vit. D levels were 3921, 3365, and 2890 ng/mL, respectively and for patients with normal Vit. D levels were 2727, 2536, and 1932 ng/mL, respectively (P=0.0211 [S]). The mean, SD, and median serum ferritin levels for patients with GI were 4643.5, 4111.22, and 3304 ng/mL, respectively and those for patients with normal serum glucose levels were 2935, 2106, and 2241 ng/mL, respectively (P=0.00977 [S]).

Discussion

β-TM is a hereditary disorder caused by a genetic deficiency in the synthesis of β-globin chains. It can affect the endocrine system, pancreas, bones, and immune

| Table 1. Clinical characteristics of patients with endocrinopathies in this study. |
|-----------------------------------|-----------------|------|------|-------------|-----------------|
| Endocrinopathy                    | Total Number (per 143 patients) | F    | M    | Mean Age | Percentage |
| Vitamin D deficiency              | 80              | 37   | 43   | 11.72     | 56%           |
| LH/FSH deficiency                 | 42              | 14   | 28   | 9.6       | 29.37%        |
| Hypothyroidism                    | 30              | 12   | 18   | 10.78     | 21%           |
| Short stature                     | 24              | 5    | 19   | 13.13     | 16.78%        |
| Growth hormone deficiency         | 18              | 3    | 15   | 12.83     | 12.58%        |
| Diabetes mellitus                 | 18              | 7    | 11   | 12.94     | 12.58%        |
| Adrenal insufficiency             | 6               | 2    | 4    | 11        | 4.19%         |
| Hypoparathyroidism                | 1               | 0    | 1    | 6         | 0.699%        |

<table>
<thead>
<tr>
<th>Table 2. Normal reference ranges for the laboratory variables reviewed in this study; these data have been obtained from King AbdulAziz University Hospital laboratory Phoenix database.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum phosphorus 0.81 - 1.58 mmol/l</td>
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<tr>
<td>Total serum calcium 2.12 - 2.52 mmol/l</td>
</tr>
<tr>
<td>Serum alkaline phosphatase enzyme 50 - 36 U/L</td>
</tr>
<tr>
<td>Serum parathyroid hormone 1.6 - 6.9 Pmol/l</td>
</tr>
<tr>
<td>Serum 25-hydroxyl vitamin D 75 - 100 nmol/l</td>
</tr>
<tr>
<td>Fasting blood glucose 3.6 - 7 mmol/l</td>
</tr>
<tr>
<td>Serum thyroid-stimulating hormone 0.27 - 1 IU/L</td>
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<tr>
<td>Serum free thyroxin 12 - 22 Pmol/l</td>
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<tr>
<td>Serum adrenocorticotropic hormone 1.1 - 3.2 Pmol/l</td>
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<tr>
<td>Morning cortisol 138 - 360 nmol/l</td>
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<tr>
<td>Serum insulin like growth factor-I level 16.6 - 85.1 ng/ml</td>
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<tr>
<td>Growth hormone stimulation test &gt; 10 ng/ml</td>
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<td>Serum luteinizing hormone 0.8 - 1.0 IU/L</td>
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<td>Serum follicle-stimulating hormone 1.6 - 1 IU/L</td>
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<td>Serum estrogen 26 - 25 Pmol/l</td>
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</tr>
<tr>
<td>Serum ferritin 30 - 400 ng/ml</td>
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system. Iron overload in organs because of numerous blood transfusions is one of the leading causes of morbidity in all patients with severe forms of β-TM. On comparing our prevalence of endocrinopathies in children and adolescents with β-TM to other studies, we found that, in recent years, several studies have reported a high incidence of endocrinopathies in children, adolescents and young adults suffering from β-TM (10). A study conducted at KAAU Hospital between 1990 and 2004 involved 360 patients with β-TM (male patients, 203; female patients, 157) the median age of the patients was 12.5 years. Endocrinopathies were found to be the second leading cause of morbidity among children with β-TM. Twenty-nine patients (10.4%) developed endocrinopathies. DM was the most common endocrinopathies (13 patients), followed by hypoparathyroidism (11 patients) and hypothyroidism (5 patients). Furthermore, serum ferritin levels were found to increase with age. In their cohort, the mean and SD serum ferritin levels were 2100, and 512.6 ng/mL, respectively for children who were 0-10 years old and 4920, and 2024.1 ng/mL, respectively among patient ages between 11_20 years old.11

An Iranian study involving 56 children who were more than 10 years of age, stated that the prevalence of endocrinopathies was as follows: DM in (8.9%), short stature (70%) in boys and (73%) in girls, hypocalcemia in (41%), primary overt hypothyroidism in (16%). Only 8 patients (14.3%) had no endocrine abnormalities. Delayed puberty was observed in approximately 71% of the patients and it was the commonest endocrinopathy, followed by short stature (51.8%). Hypoparathyroidism is thought to be a rare complication that is usually, but not always, accompanied by hypocalcemia.12 A North America study involving 361 patients who had β-TM and were above 6 years of age showed that such patients had a higher rate of multiple endocrinopathies. Hypogonadism was the most frequent endocrinopathy and affected individuals of both genders; it was observed in 14.3% of the female patients and 25.5% of the male patients. GH deficiency was present in 9.6%, DM was in 14%, and 12% of subjects were Vit. D deficient.13

A Taiwanese study was performed on 82 patients whose age was 2 years or older and were receiving frequent transfusions, (15 ml packed erythrocytes per kg body wt given every 2–3 weeks to keep their hemoglobin level at a minimum of 10 g/dl before each transfusion), found that, the prevalence of impaired glucose tolerance was (8.5%), 7 of 82, and that of DM was (19.5%), 16 of 82. Presentation with diabetic ketoacidosis was (31.1%), 5 out of 16, and (72.0%) had normal glucose tolerance. In the previously mentioned study, the incidence of diabetes was higher than in the current study (12.58%). In an Australian study all the subjects had at least 1 endocrinopathy, with 16 patients (55%) having 3 or more endocrinopathies. Hypogonadism was the most prevalent followed by osteoporosis and growth failure (less than 3rd centile) with a frequency of 16/29 (55%), 14/29 (48%) and 10/29 (35%) patients, respectively.15 Thirty seven patients, ages ≥14 years, with β-TM were studied in Turkey for endocrinopathy they concluded that (40%) of the patients had growth retardation. Gonadal dysfunction was detected in (47%) of patients. Hypothyroidism was observed in (16%) of patients. While, impaired glucose tolerance was observed in (10.8%) of patients.16

In a French study involving 267 patients with β-TM (median age, 20 years), 6% of the patients developed DM, 10% developed hypothyroidism, and 48% developed hypogonadism. The median height was also negatively affected in the youngest patients, and hypogonadism was the most frequent complication. The study conducted in France had a higher incidence of hypogonadism when compared to the presented study (21%), the incidence of both hypothyroidism and DM was less. A study conducted at the University of Ioannina in Greece involved 27 patients with β-TM who were segregated into 2 groups: group A with 15 patients aged 5-10 years, and group B with 22 patients with aged 11-23 years. The serum of 24,25-dihydroxyvitamin D concentrations of the patients with β-TM in both age groups were found to be low.18

In the current study, we compared the serum ferritin levels of unaffected and affected children and adolescents with respect to multiple endocrinopathies. We found that the serum ferritin levels of children and adolescents with delayed bone age were significantly higher than those of children and adolescents with normal bone age. Furthermore, a similar finding was observed in connection with Vit. D deficiency; the mean and median serum ferritin of children and adolescents with low Vit. D levels were higher than those of children and adolescents who had normal Vit. D levels. Iron overload and GI were found to be correlated; the serum ferritin levels of children and adolescents with GI were higher than those of children and adolescents who did not have GI.

Vit. D deficiency was the most frequent endocrine complication of iron overload in patients with β-TM, followed by delayed puberty, hypothyroidism, short stature, DM, and GH deficiency. Adrenal insufficiency and hypoparathyroidism accounted for less than 5%
of the endocrinopathies observed in patients with β-TM in the current study. In our cohort, the serum ferritin levels increased with age, which was similar to the findings obtained in other published studies.11 Furthermore, we observed similar finding with respect to the above mentioned studies in most aspects save one, a very high incidence of Vit. D deficiency due to poor appetite, decreased consumption of dairy products and lack of sun exposure. Given the high prevalence of Vit. D deficiency in the general population and the added burden of increased metabolic demands, chronic medical care, and ironoverload, it is not surprising that Vit. D deficiency is quite common among patients with β-TM.18,19 We emphasize on the importance of establishing an endocrine evidence based practice guidelines for β-TM patients, that would perform routine physical and laboratory examinations on β-TM patients. Such guidelines will improve both the morbidity and mortality rates of β-TM patients who have endocrinopathies and promote compliance to medication and life style improvements.

Conclusion
The current study provides evidence that endocrinopathies frequently occur in patients with β-TM and start early in childhood. We have identified children and adolescents with β-TM as a particularly vulnerable group for multiple endocrinopathies. Vit. D deficiency, delayed puberty, hypothyroidism, and short stature were the prevalent endocrinopathies in patients with β-TM. We emphasize on the importance of screening for endocrinopathies in children with β-TM, to prevent lifelong complications. Furthermore, we recommend that prophylactic Vit. D supplements be given to children with β-TM and that a healthier life style be promoted by encouraging sun exposure and a high intake of fortified dairy products to improve their overall Vit. D status.

Acknowledgments
The authors are grateful for the essential cooperation of both the Pediatric Department and the Hematology Department at KAAU. We would like to express our gratitude to both Dr. Mohamad Qari, head of the Hematology Department at KAAU and Dr. Yutin Al Hayes, hematology consultant at KAAU for their valued revision of the current study. In addition, we sincerely thank Dr. Areej Al Hasmi and Dr. Mohamad Al Mahayani for their participation in data collection and assessment. We highly appreciate the assistance of the nurses at KAAU who helped us in data collection and without whom this study would not have been possible.

References
Evaluation of the Laboratory tests used in the Identification of Lupus Anticoagulants

Background: Lupus anticoagulant (LA) refers to a group of autoantibodies that inhibit certain phospholipid-dependent coagulation reactions, and typically cause prolongation of activated partial thromboplastin time (APTT). These are diverse laboratory tests for antiphospholipid antibodies (APAs). This situation is compounded further by the lack of a golden standard for their detection and this has resulted in wide variation in LA testing between laboratories. The aim of this study is to evaluate the sensitivity of a wide range of assay procedures and reagents in common use for the detection of LA in patients with recurrent fetal loss.

Patients: Citrated blood samples were collected from 110 women with recurrent fetal loss (RFL) attending a special outpatient RFL Clinic, King Khalid University Hospital, Riyadh. They had history of 3 or more consecutive spontaneous unexplained abortions before the 10th week of gestation. Their ages ranged from 20 to 43 years (mean=30±7.5). Control group: 110 normal healthy Blood Donors.

Blood sample processing: Citrated blood samples were subjected to double 15 min centrifugation at 3000 rpm. The resulting platelet poor plasma (PPP) was either tested immediately or stored in aliquots in the frozen state at -40°C for testing later.

Results of Laboratory Tests used for Detection LA: the detection rates for LA among RFL patients versus healthy controls are as follows:

* The activated partial thromboplastin time (APTT, Manchester Reagent) (17.3% vs. 3.6%)
  * Mixing studies either with normal plasma (NP) or the platelet neutralization procedure (PNP):
    * Prolonged APTT + NP: (11% vs. 2.7%);
    * Prolonged APTT + PNP: (6.3% vs. 1.8%)
* The Staclot-LA test kit (Diagnostica Stago, France) (25.5% vs. 6.3%);
* PTT-LA (Diagnostica Stago, France); (4.5% vs 4.5%);
* The Kaolin Clotting Time (KCT) (28.1% vs. 1.8%);
* The Dilute Russell’s Viper Venom Test (dRVVT), (35.5% vs. 6.3%).

Conclusions: The dRVVT followed by the KCT identified more patients with LAC among those with RFL than the other tests, particularly the low-phospholipid APTT (Manchester Reagent). Staclot LA is a complete system of confirmatory and screening tests. Staclot LA is easy to perform and commercially available as a complete test system, containing both testing and confirming for LA. PTT-LA is least sensitive for the detection of LAC. The other more feasible confirmatory test is PNP combined with a sensitive APTT reagent. Depending on the available financial resources, laboratories may follow different practices in diagnosing LAC ranging from one test to combinations test procedures.
A ntiphospholipid antibodies (APAs) are a heterogeneous group of antibodies directed against complexes of phospholipids and proteins. Over the years, 2 laboratory procedures have been carried out for the detection of APAs, anticardiolipin antibodies (ACAs), and the lupus anticoagulant (LAC). Both these procedures have assumed increasing importance and popularity in view of the established association between APAs and many disease states, particularly fetal loss, thrombocytopenia, and venous and arterial thrombosis.1,2

The diversity of these antibodies and their association with a wide variety of diseases stimulated multidisciplinary interest in linking them with potential pathophysiologic mechanisms3 and generated wide interest in their utility as diagnostic tools. This has resulted in an increasing burden on hospital laboratories as tests for APAs, and particularly LAC, are more frequently ordered than before by clinicians of diverse specialties.

Lupus anticoagulant belongs to a heterogeneous group of immunoglobulins, of the IgG class, IgM class, or a combination of both the classes, directed against negatively charged phospholipids.3,4 It is an autoantibody that interferes with one or more phospholipids-dependent steps of blood coagulation, resulting in the prolongation of the prothrombin time (PT), activated partial thromboplastin time (APTT), kaolin clotting time (KCT), and diluted Russell’s Viper Venom Time (dRVVT). This prolongation is attributed to the agglutination of phospholipids present in plasma by LAC, thereby preventing their participation as cofactors in coagulation activation pathways, particularly the inhibition of the conversion of prothrombin to thrombin.5

The anticoagulant activity of these antibodies and the prolongation of in vitro phospholipid-dependent clotting tests are currently tested for by lowering the phospholipid concentration in these phospholipid-dependant tests.

The assay procedures and reagents used for the detection of APAs are on the increase and are very diverse, and given the heterogeneity of APAs, no single test is exclusively sensitive or specific for LAC. This situation is compounded further by the lack of a “golden standard” for their detection.

Therefore, there is need to throw more light on the currently available diagnostic tests for LAC, and this leaves the door open for comparative studies on the various testing procedures currently used for the detection of LAC.

Therefore, the aim of this study included the following: (i) to evaluate the sensitivity of a wide range of assay procedures and reagents in common use for the detection of LAC in patients with recurrent fetal loss (RFL) and in normal healthy controls; (ii) to find out whether one or more tests need to be undertaken to avoid missing cases positive for LAC.

Materials and Methods

Selection of subjects: Hundred-and-ten women with recurrent fetal loss (RFL) were recruited consecutively from the attendants to a special outpatient RFL clinic, King Khalid University Hospital, Riyadh, from January to December 2006. They had a history of 3 or more consecutive spontaneous abortions before the 10th week of gestation. No obvious causes of abortion (anatomical, genetic infective, etc.) were identified i.e., they were considered to have unexplained RFL. Their ages ranged from 20 to 43 years (mean=30±7.5).

Patients were tested at least 3 months after the last abortion or fetal loss. They underwent general medical examination and extensive relevant laboratory and radiological tests before enrollment as unexplained fetal loss patients.

Control group: A total of 110 normal healthy blood donors (male n=100, female n=10) from the Blood Bank Donation Center at King Khalid University Hospital, Riyadh, were randomly selected. They were taking no medication. Their ages ranged from 18 to 52 years (±SD: 31±8.2).

Blood sampling and processing: Venous blood samples were collected in sodium citrate (0.11 M) to give a blood citrate ratio of 9:1. Samples were transported without delay to the Coagulation Research Laboratory, College of Medicine. A 15-min double centrifugation at 3000 rpm was done before separating the plasma by double centrifugation as follows: (1) citrated blood was centrifuged at 3000 rpm for 15 min. (2) A plastic transfer pipette was then used to remove the supernatant platelet rich plasma, which was placed in a second plastic tube and recentrifuged under identical conditions. (3) The resulting platelet poor plasma (PPP) was either tested immediately or stored in aliquots in the frozen state at -40°C for testing later.

Assay techniques: Assay techniques for detecting lupus anticoagulant closely followed the guidelines of the Scientific and Standardization Committee of the International Society for Thrombosis and Haemostasis (ISTH), with respect to both pre-analytical and analytical variables.6
The activated partial thromboplastin time (APTT) test was performed using a sensitive low phospholipid APTT reagent (APTT Manchester Comparative Reagents–UK, Activated PTT Kaolin; Catalogue Code: R9) according to the manufacturer’s instructions. The clotting time was recorded in duplicates using a coagulometer (Start Coagulometer, Diagnostica Stago; France). After the test is taken, LA is suspected if the patient’s APTT is more than 2 SDs (3.5 s) above the mean (48±7 s) of the normal range for healthy population, which is 55 (48+7=55 s). Mixing with normal pool plasma (NP) was undertaken in samples showing >5 s prolongation of the APTT; and those samples, which did not show correction of prolongation, were tested further with the platelet neutralization procedure (PNP) to confirm the presence of LAC.

The Staclot LA: The Staclot-LA test kit (Diagnostica Stago; France) is a reagent system designed for the qualitative detection of lupus anticoagulant (LAC) in the plasma by the use of hexagonal (II) phase molecules. The Staclot LA is an APTT-based assay that is based on the principle that LAC can be neutralized by hexagonal phase phospholipids (HPP). The Haemostasis Thrombosis Laboratory uses this test to confirm the presence of a LAC following the finding of a prolonged PTT-LA that does not correct fully with normal plasma. The test plasma is incubated with and without addition of HPP. An APTT is then performed on both samples using an LA-sensitive reagent. If LAC is present, it is neutralized by HPP resulting in a shorter clotting time than the plasma without HPP. The Staclot LA is considered to be positive for the presence LAC if the difference in the clotting time between the 2 tubes is more than 8.0 s.

In 1993, Douglas Triplett and his colleagues evaluated the Staclot LA®, which utilized hexagonal (II) phase (e.g., phosphatidyethanolamine) as a confirmatory test for LAC. The Staclot LA test was performed according to the instructions provided by the manufacturer.

PTT-LA (Diagnostica Stago; France): The PTT-LA kit is intended for the determination of lupus anticoagulant (LAC) in the plasma by the use of hexagonal (II) phase molecules. The Staclot LA is an APTT-based assay that is based on the principle that LAC can be neutralized by hexagonal phase phospholipids (HPP). The Haemostasis Thrombosis Laboratory uses this test to confirm the presence of a LAC following the finding of a prolonged PTT-LA that does not correct fully with normal plasma. The test plasma is incubated with and without addition of HPP. An APTT is then performed on both samples using an LA-sensitive reagent. If LAC is present, it is neutralized by HPP resulting in a shorter clotting time than the plasma without HPP. The Staclot LA is considered to be positive for the presence LAC if the difference in the clotting time between the 2 tubes is more than 8.0 s.

Test principle: LA exerts an inhibitory effect on phospholipids, which are normally required in clotting tests, such as the APTT. The principle of the PTT-LA test is based on the measurement of plasma recalcification time in the presence of cephalin and activator silica. The presence in the test plasma of LAC prolongs the clotting time. Sensitization of the reagent, which depends on the concentration and the source of the phospholipids and the activator, specifically enhance the prolongation of the clotting time due to the LA in the test plasma.

The Kaolin Clotting Time (KCT) test was performed according to the method proposed by Extner et al.12 The KCT is an assay similar to APTT; however, exogenous phospholipid is not added to the reaction mixture, and the assay depends on the APAs to block the availability of trace quantities of phospholipid present in the centrifuged plasma. Some authors believe that KCT depends on prothrombin as a cofactor, than the dRVVT test, which appears to be more dependent on βGP1.15

The results were expressed by a calculated index according to the formula:

\[
\frac{b-c}{a} \times 100 = \text{ILA (index of lupus anticoagulant LAC)}
\]

Where \(a = \text{KCT of patient plasma (PP)}\)

\(b = \text{KCT of mixture PP + Normal Pooled Plasma (NP)}\)

\(c = \text{KCT of NP}\)

An index of 10% or more is considered positive for the presence of LAC.

Confirmatory tests

APTT mixing study:
The first step investigating prolonged APTT is to perform 1:1 mixing study of normal pool plasma (NP) with the patient test plasma. The APTT is repeated, and if the result shows APTT within normal range, a factor deficiency is suggested; if “correction” is incomplete, an inhibitor is suspected.14

The Platelet Neutralization Procedure (PNP)7 was performed in conjunction with APTT. Shortening of the APTT by <5 s in the reaction mixture as compared to the saline control is considered confirmatory of positive for LAC. The results of all the tests were recorded in duplicates.

The Dilute Russel's Viper Venom Test (dRVVT) was performed according to the method of Thiagarajan et al.15 The venom (Manchester Comparative Reagents; UK) was reconstituted using tris buffered saline, pH 7.5. Thiagarajan et al. (1986) were the first to describe the use of a modified dRVVT for the diagnosis of LAC. Their studies found the dRVVT to be more sensitive than APTT and the tissue thromboplastin inhibition test.

The principle of the assay is based on the use of snake venom to activate factor X and to initiate the co-
agulation cascade in the common pathway in the presence of a low concentration of phospholipids. This eliminates any problems, including inhibitor or factor deficiency that occurs prior to factor X activation. The dRVVT may be prolonged as a result of heparin therapy, coumarin, or decreased levels of V and X. The confirmatory test uses a high concentration of phospholipids, which should shorten the clotting time. The dRVVT has become a very popular screening test in both USA and Europe. It was performed by adding snake venom (Russell’s viper venom) to citrated plasma and recording the clotting time. LA will prolong the dRVVT by interfering with the assembly of prothrombinase complex.16

The result is taken as positive for lupus anticoagulant if the index of patient clotting time divided by control plasma clotting time is >1.1.

Statistical analysis: The Chi-square test was employed to determine the significance of differences in the prevalence of LA, based on the results of different laboratory tests.

Results

The Laboratory detection of LAC
To facilitate the comparison between the sensitivities of various assay procedures, the results for both RFL patients as well as healthy controls (blood donors) will be presented as a frequency (number and percent) of positive results of each LAC test procedure.

The screening tests:

APTT
In the first group of tests, which are based on the APTT, we used 3 different reagents APTT, (Manchester Comparative Reagent; UK), PTT-LA (Diagnostica Stago; France), and Staclot LA (Diagnostica Stago; France), whose manufacturers claim their high sensitivity for the detection of LAC.

Normal Healthy Controls
The results obtained for both healthy controls (Blood Donors n=110) and in women with history of RFL (n = 110) are shown in Table-1. In the control subjects, Staclot LA gave the highest frequency of positive results (7 out of 110; 6.3%), which is significantly higher than the results given by the APTT (Manchester) (3.6%), and the PTT-LA (4.5%).

RFL Patients
The Staclot LA gave the highest frequency of positive results (25.5%), while the APTT Manchester gave a frequency of 17.3%. The PTT LA proved to be the least sensitive as it gave a frequency of 4.5%, which was similar to that of the controls (Table 1).

KCT
The KCT gave positive results in 28.1% of the RFL patients and in 1.8% of healthy controls (Table-1).

dRVVT
The dRVVT gave the highest frequency of positive results (35.5%) in RFL patients and a frequency of 6.3% in controls, which is similar to that given by Staclot LA (6.3%). The results of the screening tests of RFL patients indicate that the sensitive test for the detection of LAC in a decreasing order of sensitivity is as follows: dRVVT, KCT, Staclot LA, the APTT Manchester, and lastly PTT-LA, while in controls the most sensitive tests were Staclot LA and dRVVT (6.3%), PTT-LA, APTT Manchester, and lastly KCT (Table 1).

The combination of the positive tests:
Despite the fact that different screening tests possibly detect different specificities of APAs, we analyzed the results to find out which combination of screening tests agree on positive results:

Staclot LA with dRVVT gave a frequency of positive results of the 14.5% in RFL patients and a frequency of 0.9% in controls. Staclot LA + KCT gave a frequency of 10% in RFL patients and 0.9% in controls; Staclot LA in combination with dRVVT + KCT gave a frequency of positive results in 7.2% of the RFL patients and in 0.9% of the controls (Table-1).

APTT Manchester + dRVVT gave the highest frequency of positive results in 15.4% of the RFL patients, which is similar to that of Staclot LA (14.5%), while the respective frequency in healthy controls is significantly lower (4.5%). On the other hand, the combination of APTT Manchester + KCT gave a frequency of positive results in 10.9% RFL patients, which is similar to that given by Staclot LA, (10%) and 1.8% in healthy controls. APTT Manchester + dRVVT + KCT gave a frequency of positive KCT results 9% of in RFL patients, which is close to that given by Staclot LA (7.2%) and 0.9% in healthy controls, which is identical to Staclot LA (0.9%) (Table 1).

APTT Manchester + NP: Only 11% (out of the 17.3% who were positive with the APTT, Table 2) were confirmed positive in RFL patients and in 2.7% (out of 3.6%, Table 2) of the healthy controls.

APTT Manchester + PNP: Only 6.3% of RFL patients were positive with the APTT Manchester, which is significantly lower than the positive results obtained with the other screening tests.
**Table 1.** The frequency of positive results of the screening tests for lupus anticoagulant (LAC), using different screening assay procedures, in patients with history of recurrent fetal loss (RFL) and healthy controls.

<table>
<thead>
<tr>
<th>Assay Procedure(s)</th>
<th>Healthy Controls (n=110)</th>
<th>RFL Patients (n=110)</th>
<th>P values</th>
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<tr>
<td></td>
<td>No. of positive Controls</td>
<td>Positive Controls %</td>
<td>No. of Positive Patients</td>
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<tr>
<td>APTT Manchester</td>
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<td>3.6%</td>
<td>19</td>
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<tr>
<td>Staclot LA</td>
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<tr>
<td>PTT-LA</td>
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<td>4.5%</td>
<td>5</td>
</tr>
<tr>
<td>KCT</td>
<td>2</td>
<td>1.8%</td>
<td>31</td>
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<tr>
<td>dRVVT</td>
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<td>6.3%</td>
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<td>0.9%</td>
<td>10</td>
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</tbody>
</table>

* The percentage positive results between patients and controls differed significantly at 5% level of significance. (n=number of observations)

**Table 2.** The frequency of positive results identified by KCT+ dRVVT, APTT+Manchester +PNP, APTT+N, and the three screening test (dRVVT+KCT+APTT).

<table>
<thead>
<tr>
<th>Combinations of assay procedures</th>
<th>Healthy Controls (n = 110)</th>
<th>RFL Patients (n= 110)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of positive Control</td>
<td>Positive Control %</td>
<td>No. of Positive Patients</td>
</tr>
<tr>
<td>KCT+dRVVT</td>
<td>2</td>
<td>1.8 %</td>
<td>9</td>
</tr>
<tr>
<td>APTT-Manchester +PNP</td>
<td>2</td>
<td>1.8 %</td>
<td>7</td>
</tr>
<tr>
<td>APTT-Manchester +NP</td>
<td>3</td>
<td>2.7 %</td>
<td>12</td>
</tr>
<tr>
<td>3 tests positive</td>
<td>2</td>
<td>1.8 %</td>
<td>14</td>
</tr>
</tbody>
</table>

* The percentage positive results between patients and controls differed significantly at 5% level of significance. (n= number of observations)

Phospholipids play multiple roles in the coagulation system as they participate at several critical points of the coagulation pathways, both extrinsic and intrinsic. In the intrinsic pathway, phospholipids are required for the activation of factor IX in the presence of FVIII (Antihaemophilic factor), platelets, and calcium ions, which in turn activates FX to active FXa. In the extrinsic pathway, tissue factor and FVII complex, in the presence of phospholipids, platelets and calcium ions, activate factor X to active FXa. In the final common pathway of coagulation, prothrombin is converted to
thrombin when bound to phospholipids. Therefore, APAs, whether LACs or ACAs, are expected to act at these numerous sites in the coagulation activation pathways, targeting membrane phospholipids as well as plasma protein co-factors.

LACs were given that name because they were originally detected in patients with SLE. Their anticoagulant property emerges from the fact that they cause prolongation of activated partial thromboplastin time (APTT). Prothrombin time (PT) reagents usually contain high concentrations of phospholipids that will mask the antigen/antibodies reaction and are rarely prolonged by LAC, whereas APTT reagent, particularly those with low concentration of phospholipids, do not bind all the circulating antibodies resulting in the prolongation of clotting times. This has led to the assumption that patients with LAC are prone to bleeding; on the contrary, LAC patients are predisposed to thrombosis.4,17

Requests for the detection of APAs are on the increase, so are the diverse laboratory assays used for their detection. This situation is compounded further by the wide diversity of these APAs and the lack of “a golden standard” for their detection, especially for the LACs. This leaves the door open for comparative studies on the available reagents and assay procedures used for the detection of LAC in the hope of identifying the most sensitive assay procedure(s), and also triggers numerous efforts to standardize the test procedures employed in the detection of LAC.

Efforts to standardize the laboratory tests for LAC date back to 1992, when an intra-laboratory survey highlighted the variation in APA testing between laboratories.18 Although the basic laboratory techniques used were the phospholipids-dependent coagulation tests, APTT, KCT, and dRVVT, most centers in this survey used only one of these assays to test for LA. In later years, many studies that dealt with the evaluation of the laboratory tests for LAC6-25 highlighted the major difficulties testing LAC, which are still with us today. Basically, the wide variation in the test procedures used results in a wide variation in the detection rate of LAC. This dilemma triggered the ISTH to take the initiative and assembled world experts in coagulation testing to work out a standard procedure for testing for LAC, by detailing all the steps from blood collection to the final step and deciding positive from negative results,6 and these guidelines were updated recently.26

Despite these guidelines and voluminous literature on LAC testing that has accumulated in the last 2 decades, we are still in a dilemma. This is best highlighted in 2 very recent North American studies. The first was published in 2010, in which a review was conducted on the performance and practices by North American clinical laboratories.7 The study found that the APPT and the dRVVT constituted major testing methods, and that LAC-sensitive APTT methods were more sensitive to weak LA than dRVVT-based methods but were less specific. In confirmatory testing, dRVVT methods performed better, but the performance was LAC dependent. Noncompliance with recommendations for LAC testing according to the International Society on Thrombosis and Haemostasis Guidelines was high (8%–38%), with the majority of noncompliant laboratories failing to report results of mixing studies. It was concluded that the survey provided new insights into LAC testing in North America and identified opportunities for standardization.

In the results of the second study,28 2 questionnaires were distributed to the clinical laboratory members of the North American Specialized Coagulation Laboratory Association (NASCOLA) and the ECAT Foundation (ECAT) to determine their LAC testing practices, and checked if they conformed with the published recommendations. The first and second questionnaires were completed by 113 and 96 laboratories, respectively. Commonly performed LAC tests included the dRVVT, LAC-sensitive APPT, and hexagonal phospholipid test. Although some laboratories did single LAC tests, the majority complied with published recommendations: (1) to use platelet poor plasma for LAC tests; (2) to use two or more screening tests representing different assay principles, and one assay having a low phospholipid concentration to exclude LAC; (3) to confirm LAC phospholipid dependency by the method giving an abnormal LAC screen; (4) to document the inhibitor activity on pooled normal plasma; (5) and not to use phospholipid antibodies to confirm LAC. A minority (<35%) followed the recommendations to exclude factor deficiencies and factor inhibitors as the cause of an abnormal LAC test. After participating, 32% of the laboratories had changed practices and 20% indicated that they would be changing practices. While most laboratories generally follow published guidelines for LAC testing, few follow recommendations to evaluate for other coagulation abnormalities.

The results obtained in the present study, which is a single institute study employing multiple procedures for testing for LAC, showed that out of the many APTT-based tests, Staclot LA is the most sensitive as it gave the highest frequency of positive results in 28 (25.5%) out of 110 RFI patients, while LAC-sensitive
APTT (Manchester) gave a frequency 17.3%, PTT-LA gave only 5 (4.5%) positive results of 110 RFL patients and failed to detect positive results, which were identified by others.

The reliability of the APTT got further support from the findings of the Working Group on Haemostasis of the Société Française de Biologie Clinique, employing the APTT reagent (Organon-Teknika), tissue thromboplastin inhibitor assay (TTI) (Thromborel Behring-werke) and Staclot LA. The study concluded that Staclot LA is the most sensitive and specific test. Similarly, Triplett et al in their study of 20 plasma samples previously identified as positive for LAC, found that all 20 samples were positive for the Staclot LA test; i.e., a sensitivity of 100% and concluded that the Staclot LA procedure has a very high sensitivity to LAC-positive plasmas.

Disagreements with the above findings have been reported. Thus, Schjetlein and Wisløff (1995) in their study of 30 known LAC positive plasma samples, found that the PTT-LA and Staclot dRVVT gave a sensitivity of 67% (20 out of 30). This disagreement can be attributed to the fact that they used known LA positive plasma samples. The determination of positive PTT-LA test was defined by the manufacturer (i.e., >47 s). We established our own reference positive value for PTT-LA, which is 55.1 s (which is the mean of normal (48.1) + 2SD). On the other hand, the APTT-Manchester reagent gave positive results with a frequency of 17.3% in RFL patients. This is in line with the results of the LAC Working Party in United Kingdom, which defined Manchester APTT as a sensitive reagent for LAC.

PTT-LA (Diagnostica Stago; France) proved to be the least sensitive reagent as it failed to detect most of positive results identified by the other reagents (Staclot LA and APTT Manchester). This agrees with the findings of Denis-Magdelaine et al and Arnout et al that the responsiveness of PTT-LA appeared to be significantly lowered when performed on the KC10 Coagulometer (Amelung Lemgo; Germany). Instrument dependency of the LAC sensitivity has not been documented in other studies.

The dRVVT produced a remarkably higher rate of positive results than other tests; 35.5% positively in 110 RFL patients as compared to 6.3% in healthy controls. This agrees with the findings of Moor et al who undertook the test in 2843 patients with a thrombembolic disease, and found a high frequency of 40.7% (417 out of 1024) of positive results. Similarly, Ferro et al studied 53 consecutive patients, with either SLE, or a history of miscarriage or both; the dRVVT gave a sensitivity of 36%. Monica et al employed 2 coagulation assays, KCT and dRVVT, on the plasma of 72 patients who were previously diagnosed positive for LAC (using Staclot LA to confirm the presence of LAC); dRVVT gave a frequency of 41% (30 out of 72 patients), whereas KCT gave a frequency of 36% (26 out of 72 patients). Pengo et al also agree that the dRVVT is the most sensitive and specific test for LAC. Similarly, Anne et al prospectively studied 584 consecutive patients who were referred to Mayo’s Special Coagulation Laboratory for either suspected APAs or unexplained prolonged clotting times at the Mayo Clinic in Rochester, Minn Esota (USA). All the patients were screened for LAC using different assay procedures, APTT reagent (BioMerieux; Durham, NC), dRVVT, and KCT. Among these, 61 patients (10.4%) were positive for LAC out of 584 patients. The dRVVT was the mostly positive LA assay (74% of the 61 patients with positive results for LA). On the other hand Rune et al studied 30 plasma samples known to be positive for LAC that belonged to patients with thromboembolic disease, pregnancy complications, or autoimmune disease; the sample was considered positive for LAC if it gave a PTT-LA clotting time <47.7 s and dRVVT <32.9 s. The dRVVT gave a high frequency of the positive results reaching 80% (24 out of 30); the limit of the clotting time for the dRVVT was 32.9 s (mean+2 SD; 27.7±2.6). In our study, we considered a positive result a ratio < 1.2.

The results of the current study have shown that KCT is less sensitive than dRVVT, but more sensitive than APTT assays (APTT Manchester, Staclot LA, and PTT-LA). It gave a positivity of 29.1% among RFL patients, compared to 1.8% normal healthy controls. This finding agrees with Ferro et al who studied 53 consecutive patients with SLE, who had a history of miscarriage or both. They found a detection rate of KCT positivity of 27% compared to a much higher rate of 36% for the dRVVT.

In RFL patients, the combination of APTT Manchester, dRVVT, and KCT gave a LAC detection rate of 9.1%, which is not markedly different from that detected by the combination of Staclot LA, KCT, and dRVVT (8.2%).

The results of the present study further confirmed the limitations inherent in the laboratory detection of LAC, which in simple term; no 2 tests seem to agree on. Given the diversity of these autoantibodies along with similar diversity in the currently available reagents and test procedures, it is left to individual laboratories to use multiple tests to improve the detection of LAC. In a recent study, it was shown clearly that the
more tests are performed; the better is the chance of identifying LAC in patient samples, since no single test has sufficient specificity and sensitivity for the detection of LAC. This could make a difference in the management of such enigmatic disease states, such as unexplained recurrent fetal loss. Finally, the new and more rigorous guidelines issued by the ISTH, if applied strictly, could be instrumental in improving the laboratory diagnosis of LAC.

In conclusion, the results obtained in the current survey on the performance of commonly available reagents and test procedures employed for the detection of LAC confirm what many other studies have already found that the dRVVT and KCT gave the highest detection rates and performed better than the low-phospholipid APTT. As expected, mixing studies whether by the normal pooled plasma or PNP procedure when used to confirm the specificity of the coagulation inhibitor reduced the detection rate. Guidelines have been proved difficult to follow, in part due to its cost and labor intensiveness. This could prove to be significance in a cost-conscious health service, particularly in private medical practice. Therefore, the conflict between the ever-improving guidelines and what it entails of excessive laboratory effort and cost will remain a critical factor in drawing the line between what is positive or negative LAC test, and accordingly whether a patient with RFL or other conditions known to be associated with LAC, particularly arterial and venous thrombosis, will or will not receive directed therapy.
LABORATORY TESTS FOR LUPUS ANTICOAGULANTS

References


Low bone mineral density in patients with sickle cell disease: Association with blunted parathyroid hormone response and accelerated bone turnover

Jalaluldin A. Jalal, Mohamed F. Elshal, Mohamed H. Qari, Maryam A. Al-Ghamdy, Amna E. Bernawi

**BACKGROUND/AIM:** Bone complications in sickle cell disease (SCD) have been well documented, but the mechanisms underlying SCD are poorly characterized. Therefore, we conducted this study to elucidate the factors affecting bone health in patients with SCD by measuring bone turnover markers and correlating them with bone mineral density (BMD).

**PATIENTS AND METHODS:** Serum from 30 patients with confirmed SCD and 20 age-matched healthy controls were included in the study. Dual X-ray absorptiometry was used to determine the BMD of the lumbar spine (L2-L4) and of the whole body.

**RESULTS:** According to the WHO definitions for T-scores, 30% of HbSS patients were osteopenic (30%) or osteoporotic (50%) in at least 1 of the 3 studied locations. BMD, serum calcium, and parathyroid hormone (PTH) showed a significant decrease, while PO4, osteocalcin (OC), and bone-specific alkaline phosphatase (b-ALP) showed a significant increase in the patient group compared to the control group (P<0.05, for all). Multivariate analysis identified only serum PTH as an independent determinant of low whole-body BMD in HbSS (P=0.0399), whereas it identified osteocalcin, magnesium, and CTX as independent predictors for PTH.

**CONCLUSIONS:** These findings suggest that low BMD is prevalent in SCD and that blunted PTH response to reduced total calcium levels and accelerated bone turnover may be the underlying mechanisms.

**KEYWORDS:** Sickle cell anemia, osteoporosis, BMD, parathyroid hormone, bone turnover markers.

Bone involvement is a frequent cause of acute morbidity in sickle cell anemia and some of its variant hemoglobinopathies. Published studies suggest that children with sickle cell disease (SCD) often have undiagnosed osteopenia or osteoporosis. Osteoporosis is defined as a state of increased fracture risk because of low bone mineral density (BMD) and deterioration in the bone microarchitecture. In patients with b-thalassemia, a red blood cell disorder characterized by anemia and low BMD, the pathophysiology of low BMD is related in part to increased bone resorption; however, the pathophysiology of similar bone manifestations in SCD is less well characterized.

A substantial body of evidence suggests that microvascular occlusion by sickled erythrocytes may cause ischemic modifications of osseous tissue and thereby lead to osteonecrosis. Moreover, impaired bone blood flow was found to increase the apoptosis of osteoblasts and osteocytes, which may lead to osteoporosis. Although osteonecrosis accounts for most chronic severe pain and further deterioration of...
the quality of life in patients with SCD, other skeletal deformities including vertebral end-plate depression, mostly due to osteoporosis, have also been reported, especially in young patients. Recent studies have acknowledged BMD as an intermediate osteoporosis marker that can be used to recognize the patient’s risk of fracture, but changes in the microarchitecture of the bones are not captured by BMD measurements. In this regard, the measurement of bone turnover markers may complement BMD testing. These markers have proven useful in screening for fracture risk in elderly patients, assessing therapeutic response to antiresorptive agents, and identifying patients with high bone turnover to predict rapid bone loss. Hormones play an essential role in skeletal health. Evidence suggests that PTH exerts anabolic action on bone partly through local growth factors and anti-apoptotic action on osteoblasts. Moreover, intermittent administration of PTH was found to be useful in the treatment of osteoporosis, since it improves bone architecture and strength without causing the appearance of abnormal bone elements. Nevertheless, scarce data exist on the relationship between PTH, bone turnover, and BMD, the gold standard measure for identifying patients at risk of osteoporosis, in particular, adults with SCD. Therefore, we aimed to investigate the possible relationships between BMD, PTH, and several bone turnover markers, including the bone formation markers osteocalcin (OC) and bone-specific alkaline phosphatase (b-ALP), and the bone resorption markers C-terminal telopeptide of type I collagen (CTX) and N-terminal telopeptide of type I collagen (NTX).

Materials and Methods
SCD patients who regularly visited the outpatient clinic of King Abdulaziz University Hospital were enrolled in the study after ethical approval was obtained from the Research and Scientific Committee of College of Medicine, King Abdulaziz University, Jeddah, KSA. Informed consent was obtained from each participant prior to enrollment. The patients had their weight and height measured to calculate the body mass index (BMI). Their history was noted, and a clinical examination was conducted, which was followed by appropriate investigations to rule out secondary osteoporosis. The following patients were excluded from the study: (1) those who were diagnosed and treated for osteopenia and osteoporosis; (2) those who were on steroids; (3) those who had anorexia nervosa, hyperthyroidism, chronic obstructive pulmonary disease, liver disease, or inflammatory bowel disease; and (4) those who had undergone organ transplantation.

Whole-body BMD and T-score were determined twice by dual X-ray absorptiometry (Hologic QDR 2000; Bedford, MA) at the Osteoporosis Center of Excellence, King Fahad Medical Research Center (KFMRC), King Abdulaziz University, Jeddah. All blood samples were collected at 10 AM. Serum samples were separated by centrifugation at 3000 rpm for 10 min and then were stored in the freezer at −20°C until analysis. The bone formation marker b-ALP was analyzed using the IDS BAP immunosay (Immunodiagnostic Systems Inc., Fountain Hills, AZ, USA). The analytical sensitivity for the BAP assay was <1 U/L (reference range, 50–136 U/L) with intra- and interassay variability lower than 10.1% and 10%, respectively. The other bone-specific marker, osteocalcin (OC), was measured using BioSource, a human Osteocalcin Enzyme Amplified Sensitivity Immunoassay (bOST)-EASIA kit (BioSource Europe S.A., Belgium). The analytical sensitivity of OC was <0.4 ng/mL (reference range, 6.8–32.2 ng/mL), with intra- and interassay coefficients of variation (CV) values of 5.2% and 6.7% respectively. Bone resorption markers included CTX, which was measured using Immunodiagnostic System Limited (Immunodiagnostic Systems Inc., Fountain Hills, AZ, USA), and NTX, which was analyzed using the Wampole Laboratories (USA). The reference range for CTX is between 0.13 and 4.1 ng/mL, and it is 10–60 nM BCE for NTX, with a CV of 4.6%.

Intact serum parathyroid hormone (iPTH) was analyzed by enzyme-linked immunosorbent assay (Immunodiagnostic Systems Inc., Fountain Hills, AZ, USA). The reference range for iPTH was 15–65 pg/mL (1.6–6.9 pmol/L), with an analytical sensitivity of <0.1 pmol/L and intra- and interassay CV values of less than 5% and 7%, respectively (Aloia et al. 2006). Serum calcium (Ca), magnesium (Mg), and inorganic phosphorus (iP) were measured using an endpoint assay in the Dade Behring Dimension-RxL Clinical Chemistry System (Dade Behring; International Incorporated, USA). The total calcium concentration was adjusted for serum albumin. The laboratory reference ranges were 2.2–2.6 mmol/L for total Ca, 0.8–1.2 mmol/L for Mg, and 1.0–1.4 mmol/L for iP.

Statistical analysis
Results are expressed as the mean±standard deviation (X ± SD). The statistical significance of the differences between groups was determined using one-way analysis of variance (ANOVA) combined with the post-ANOVA Tukey-Kramer test for multiple com-
comparisons. Uni- and multivariate regression analyses were performed to evaluate the strength of the relationship between BMD and other studied parameters. $P<0.05$ was defined as statistically significant. A sample size analysis demonstrated that 19 subjects would be required in each group to detect a statistically significant difference of $P<0.05$ in BMD between groups (one SD of 0.13; power, 88%). Data were analyzed using SPSS software version 13.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Thirty patients with SCD (19 female and 11 male) were included in the study; 20 age-matched healthy volunteers (11 female and 9 male) were included in the control group. There were no significant age or sex differences between the patients and the individuals in the control group. However, the BMD results demonstrated that SCD patients showed a significant reduction of 17.6%, 15.45%, and 12.3% at L2, L4, and for the whole body in comparison with those of the healthy control group, respectively ($P<0.001$; Table 1).

There was also a highly statistically significant difference between the groups for the T-scores at L2 and for the whole body, and a more modest one for the T-score at L4. For all the T-scores, the values were lower in the SCD group than in the control group (Table 1). Cases studied were classified into normal BMD (T-score greater than –1 SD), osteopenia (T-score greater than –2.5 SD and less than –1 SD), and osteoporosis (T-score less than –2.5 SD) according to the guidelines proposed by the World Health Organization (WHO) for the diagnosis of osteoporosis based on measurement of BMD.$^{24}$ In general, the T-scores showed that 30% of the patients were osteopenic and 50% were osteoporotic in at least 1 of the 3 studied locations. In contrast, the T-scores of the control subjects were within normal range (T-score $>-1$).

As shown in Table 2, SCD patients had low, borderline serum Ca levels (2.2±0.39 mmol/L) and showed a significant increase in serum phosphorus levels compared to the control group. There were no significant differences in serum magnesium levels between the 2 groups. A highly significant decrease ($P<0.0001$) in serum PTH was found in the HbSS patients relative to the controls (Table 1).

The biochemical bone markers results showed a significant increase in the levels of the bone formation markers $b$-ALP and OC in HbSS patients when compared to controls; however, the CTX and NTX levels were not significantly different between the groups (Table 1). In terms of the correlation between PTH and the other studied parameters, BMD and osteocalcin showed a significantly positive correlation ($r=0.387$, $P<0.008$; $r=0.585$, $P=0.0001$, respectively), whereas PTH showed a significantly negative correlation with CTX ($r=0.343$, $P<0.05$). Both PTH and BMD also correlated negatively with serum phosphorus levels ($r=0.447$, $P<0.0001$; $r=0.373$, $P<0.01$ respectively) (Table 2).

The results of the multiple regression analyses with BMD as the dependent variable are presented in Table 3. Stepwise multiple regression analyses revealed that PTH was an independent determinant of BMD ($\beta=0.045$, $P=0.0399$). Multiple linear regression analyses indicated a significant correlation of PTH with osteocalcin, magnesium, and CTX.

Discussion

Beyond the hematological severity and heterogeneity of SCD,$^{25}$ bone and joint complications are the most common manifestations.$^{25}$ These complications include bone infarction, osteomyelitis, osteonecrosis, osteopenia, and osteoporosis.$^{34}$ Accelerated hemopoiesis and bone infarction probably contributed to the low BMD in these patients.$^{26}$ Low BMD is a hallmark of osteoporosis and increased fracture risk.$^{27}$ In the present study, patients with SCD had a significantly lower BMD concentration at L2 ($P<0.001$) and L4 ($P<0.05$), as well as low borderline serum Ca levels ($2.2\pm0.39$ mmol/L). Low serum calcium levels in SCD patients have been reported in earlier studies$^{30,31}$ and have been suggested to be a result of impairment of the intestinal absorption of calcium in SCD patients.$^{30}$ In the present study, serum calcium showed positive correlation with serum phosphorus and magnesium ($r=0.390$, $P<0.032$; $r=0.736$, $P<0.0001$, respectively).
Table 1. Characteristics and laboratory data of healthy controls and of sickle cell patients in steady state.

<table>
<thead>
<tr>
<th></th>
<th>SCD group</th>
<th>Control group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=30)</td>
<td>(n=20)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>*22.37±7.40</td>
<td>22.87±4.91</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Gender (m:f)</td>
<td>11:19</td>
<td>9:11</td>
<td></td>
</tr>
<tr>
<td>BMD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L2 (gm/cm²)</td>
<td>0.89±0.32</td>
<td>1.08±0.137</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>L4 (gm/cm²)</td>
<td>0.93±0.28</td>
<td>1.10±0.15</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Total body (gm/cm²)</td>
<td>1.00±0.13</td>
<td>1.14±0.09</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>T-score</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>L2</td>
<td>-2.15±1.91</td>
<td>-0.97±1.18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>L4</td>
<td>-2.06±2.00</td>
<td>-0.78±1.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Total body</td>
<td>-2.22±1.02</td>
<td>-0.75±1.05</td>
<td>&lt;0.001</td>
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<tr>
<td>Bone Markers</td>
<td></td>
<td></td>
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<tr>
<td>Ca (mmol/L)</td>
<td>2.21±0.39</td>
<td>2.36±0.09</td>
<td>&lt;0.05</td>
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<tr>
<td>Mg (mmol/L)</td>
<td>0.84±0.14</td>
<td>0.82±0.07</td>
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<td>iP (mmol/L)</td>
<td>1.30±0.25</td>
<td>1.14±0.23</td>
<td>&lt;0.05</td>
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<tr>
<td>PTH (pmol/L)</td>
<td>2.22±1.26</td>
<td>4.11±1.46</td>
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<tr>
<td>Bone Turnover Markers</td>
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<tr>
<td>b-ALP (U/L)</td>
<td>77.13±21.50</td>
<td>64.65±21.51</td>
<td>&lt;0.05</td>
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<tr>
<td>OC (ng/ml)</td>
<td>59.03±13.01</td>
<td>43.06±9.04</td>
<td>&lt;0.05</td>
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<tr>
<td>CTX (ng/ml)</td>
<td>0.41±0.16</td>
<td>0.36±0.16</td>
<td>&gt;0.05</td>
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<tr>
<td>NTX (ng/ml)</td>
<td>1.06±0.41</td>
<td>0.97±0.36</td>
<td>&gt;0.05</td>
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</tbody>
</table>

* Mean±SD. Ca: calcium, Mg: magnesium, iP: inorganic phosphorus, b-ALP: Bone alkaline phosphatase; OC: total osteocalcin; CTX-I: C-terminal telopeptide of type-I collagen; NTX: N-terminal telopeptide of type-I collagen.

Table 2. Univariate analyses of relationships between BMD and other markers of bone metabolism.

<table>
<thead>
<tr>
<th></th>
<th>BMD *r</th>
<th>P value</th>
<th>Ca *r</th>
<th>P value</th>
<th>PTH r</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMD</td>
<td>1.000</td>
<td>--</td>
<td>0.211</td>
<td>0.300</td>
<td>0.329</td>
<td>0.042</td>
</tr>
<tr>
<td>Ca</td>
<td>0.211</td>
<td>0.300</td>
<td>1.000</td>
<td>--</td>
<td>0.231</td>
<td>0.247</td>
</tr>
<tr>
<td>PTH</td>
<td>0.329</td>
<td>0.042</td>
<td>0.231</td>
<td>0.247</td>
<td>1.000</td>
<td>--</td>
</tr>
<tr>
<td>Mg</td>
<td>0.209</td>
<td>0.390</td>
<td>0.269</td>
<td>0.167</td>
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<td>0.167</td>
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<td>0.761</td>
<td>0.550</td>
<td>0.002</td>
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<td>-0.289</td>
<td>0.268</td>
<td>0.168</td>
<td>0.083</td>
<td>0.674</td>
<td></td>
</tr>
<tr>
<td>NTX</td>
<td>-0.097</td>
<td>0.610</td>
<td>0.268</td>
<td>0.168</td>
<td>0.083</td>
<td>0.674</td>
</tr>
</tbody>
</table>

*Pearson Correlation coefficient, Sig. (2-tailed). Abbreviations as in Table 1.
On the other hand, our finding of decreased PTH in SCD contradicts the results of Mohammed et al. These conflicting data may be a result of subject selection. In his study, Mohammed et al noticed that PTH was significantly higher in the patients, with 31% having values above the normal range. In our study, PTH levels were within the low average to borderline ranges (2.2±1.26 pmol/L), with 30% of patients showing PTH concentrations below the normal range (1.6–6.9 pmol/L). Previous studies have attributed the reduced PTH levels in patients to various factors, including different types of hemoglobinopathies and iron overload, as secondary to repeated blood transfusions. Iron overload was found to exert oxidative stress and to result in signs of toxicity and dysfunction for the vast majority of endocrine glands, including the parathyroid gland. Although most of our patients had received frequent blood transfusions as indicated in their medical records, we did not evaluate this possibility, as the assessment of iron overload constitutes a diagnostic challenge owing to the unreliability of serum ferritin levels and the risks associated with liver biopsy in patients with SCD. In addition, studies involving SCD have not shown any agreement on the relationship between ferritin and BMD.

PTH deficiency may occur when parathyroid gland tissue has been traumatized or its blood supply has been interrupted. Other causes include immune-mediated destruction, congenital hypoplasia or aplasia, and end-organ resistance. Blunted PTH response to the correction in reduced calcium levels, acting secondary to magnesium deficiency, may be another possible explanation. In osteoporotic patients, it was found that hypomagnesemia is linked to vitamin D deficiency and can blunt the PTH response. In our study, although there was no significant difference in serum magnesium concentrations between SCD patients and healthy controls, 30% of the SCD patients showed magnesium concentrations lower than the normal range (0.7–1.0 mmol/L). Moreover, magnesium showed a significantly positive correlation with PTH (r=0.436, P<0.02) and Ca (r=0.329, P<0.05). This result is in agreement with that of Olukoga and coworkers, who reported low plasma magnesium levels in SCD. In addition, low PTH levels have been reported by other investigators studying impaired parathyroid gland function in magnesium deficiency.

PTH has anabolic actions on bones as it acts on the proliferative capacity of osteoblastic cells. Osteoblasts were found to not only play an important role in bone formation, but also to stimulate osteo-

---

**Table 3. Multivariate analysis of relationships between either BMD or PTH with other studied parameters.**

<table>
<thead>
<tr>
<th></th>
<th>Bone Mineral Density (BMD)</th>
<th>Parathyroid hormone (PTH)</th>
<th>Calcium (Ca)</th>
<th>Magnesium (Mg)</th>
<th>Phosphorous (P)</th>
<th>Osteocalcin (OC)</th>
<th>Bone specific alkaline phosphatase (b-ALP)</th>
<th>C-terminal telopeptides of type I collagen (CTX)</th>
<th>N-terminal telopeptide of type I collagen (NTX)</th>
<th>Age</th>
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</thead>
<tbody>
<tr>
<td>Beta coefficient</td>
<td>0.145</td>
<td>0.04</td>
<td>-0.062</td>
<td>0.397</td>
<td>-0.123</td>
<td>-0.026</td>
<td>0.013</td>
<td>-0.131</td>
<td>0.013</td>
<td>0.079</td>
</tr>
<tr>
<td>P value</td>
<td>0.373</td>
<td>0.04</td>
<td>0.784</td>
<td>0.012</td>
<td>0.013</td>
<td>0.94</td>
<td>0.94</td>
<td>0.013</td>
<td>0.918</td>
<td></td>
</tr>
</tbody>
</table>

Beta-coefficient: standardized regression coefficient representing the independent correlation between the respective variable and the dependent variable (after controlling for all other independent variables studied).

*Dependent Variable: BMD, Predictor in the Model: PTH. *Dependent Variable: PTH, Predictors in the Model: osteocalcin, magnesium, CTX.
Blunted PTH response and low BMD in sickle cell disease

Clastic bone resorption under the action of PTH. Bone remodeling in healthy adults is based on the balanced actions between osteoclasts resorbing old bones and osteoblasts forming new ones. An imbalance in this process, which occurs whenever bone resorption exceeds bone formation, causes osteoporosis. To validate these data in our study, we measured bone-specific alkaline phosphatase (b-ALP) and OC, phenotypic markers for osteoblasts in the early stage of differentiation and terminally differentiated osteoblasts, respectively. It was also found that both b-ALP and OC were significantly increased in the SCD group compared with the control (\(P<0.05\)). These findings are in agreement with previous studies. In addition, Wong et al found that OC was negatively correlated with cortical BMD. Our result is in line with this study, since OC, but not b-ALP, showed a significant but inverse correlation with BMD (\(r=-0.348, P<0.05\)). Furthermore, PTH correlated positively with OC (\(r=0.550, P=0.002\)), but not with b-ALP.

The bone resorption markers CTX and NTX, which are released during the bone resorption process, were found to be increased in the patient group compared to the control group; however, none of these markers disclosed a statistically significant difference between the 2 groups. These elevations in the bone turnover markers in our patients lead us to suggest that the overall bone turnover is increased in SCD as a compensatory reaction to low BMD in patients with SCD.

In conclusion, the current findings demonstrate that low BMD is prevalent in SCD and that blunted PTH response to reduced total calcium levels and accelerated bone turnover may be the underlying mechanisms. Moreover, our study points out a characteristic profile of SCD and stresses the great importance of monitoring serum magnesium concentrations and investigating the causes of its deficiency as a possible pathophysiological etiology of blunted PTH response and low BMD in SCD.
References

Heparin is an important anticoagulant drug widely used to treat and prevent thromboembolism. Heparin-induced thrombocytopenia (HIT) is a major complication of heparin therapy, with an incidence of 1–5% in patients treated using heparin. There are 2 types of HIT: type 1 and type 2. Type 1 presents within the first 2 days after heparin exposure, and the platelet count normalizes with continued heparin therapy; type I HIT is a non-immune disorder caused by the direct effect of heparin on platelet activation. Type 2 HIT is an immune-mediated disorder that typically occurs 4–10 days after heparin exposure and has a more serious effect than does Type 1 HIT, with grave thrombotic complications. Because of the high morbidity and mortality associated with HIT II, an immediate change of the heparin derivative is indicated. This change is sometimes difficult because of the absence of available alternatives or the occurrence of clinical conditions, such as renal failure, that may compromise the use of alternative anticoagulation drugs. Laboratory data supporting the clinical suspicion are very helpful for making this deci-
test validation

sion. Various laboratory tests have been examined as a tool for confirming the suspicion of Type 2 HIT. In this study, we validated a monospecific platelet factor 4 (PF4) immunoglobulin G (IgG) assay; this assay is a qualitative screening assay for detecting heparin-associated IgG antibodies in human serum.

Methods

A cohort of 100 patients for whom HIT was suspected was included in the study. Blood samples were obtained from 20 patients who had positive clinical pretest probability scores. The samples were collected without anticoagulant by using an aseptic technique and were aliquoted for the particle gel immunoassay (PaGIA; Diamed ID, Switzerland), enzyme-linked immunosorbent assay (ELISA) (heparin/PF4 [HPF4] Stago, Asnières sur Seine, France), and monospecific ELISA (GTI, USA) or were frozen at −70°C or below and were sent to a reference laboratory for the serotonin-release test. Complete blood count results and the clinical probability score was obtained for each patient.

Quality control for PF4 IgG is built into the test system by the inclusion of positive and negative serum controls. These controls were included in each test run to help determine whether technical errors or reagent failure had occurred. For ELISA, the mean optical density for the negative control was ≤0.300 and for the positive control was ≥1.800. Patient serum was added to microwells coated with PF4 complexed with polyvinyl sulfonate (PVS). Binding occurred if an antibody that recognized a site on PF4:PVS was present. Unbound antibodies were washed away. An alkaline phosphatase-labeled anti-human serum globulin reagent (anti-IgG) was added to the wells and incubated. The unbound anti-IgG was washed away, and the substrate p-nitrophenyl phosphate (PNPP) was added and incubated for 30 minutes. The test result

Table 1. Results of the four methods used for validation.

<table>
<thead>
<tr>
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<tbody>
<tr>
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<td>Negative</td>
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<td>4</td>
<td>Negative</td>
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<td>5</td>
<td>Negative</td>
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<td>Negative</td>
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<td>6</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
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<tr>
<td>7</td>
<td>Weak Positive</td>
<td>Weak Positive</td>
<td>Negative</td>
<td>Borderline Positive</td>
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<tr>
<td>8</td>
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<td>Negative</td>
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<td>Negative</td>
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<td>19</td>
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<tr>
<td>20</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>
was reported as positive if the OD value was equal to or greater than 0.400.

The test has the following limitations: Erroneous results may be obtained in the case of bacterial contamination of test materials, inadequate incubation periods, inadequate washing and decanting of test wells, exposure of substrate to stray light, omission of test reagents, exposure to temperatures higher or lower than those prescribed, or omission of steps. The presence of immune complexes or other immunoglobulin aggregates in the patient sample may cause increased nonspecific binding and lead to false-positive results in this assay. The results of this assay should not be used as the sole basis for a clinical decision but should be correlated with clinical conditions.

Results

Of the 20 samples tested, 1 was clearly positive and another one had borderline positivity in the serotonin-release test. The results of all 4 tests were in agreement for all samples—except for the sample with borderline positivity, which was missed by ELISA (STAGO). The frequency of samples positive for HIT antibodies was 10%. The results have been provided in Table 1.

References

A 20-year-old man presented with a 2-month history of fever, epistaxis, weight loss, and progressive hearing loss. His initial workup showed pancytopenia.

Physical examination revealed no abnormalities except for polydactyly of the left hand. His complete blood count (CBC) showed a white blood cell (WBC) count of 1.46 × 10⁹/L (normal 3.90–11.00 × 10⁹/L); hemoglobin, 86 g/L; platelet, 62 (150–350 × 10⁹/L); polymorph, 1%; lymphocytes, 88%; blasts, 2%; monocytes, 4%; atypical lymphocytes, 5%; and basophil, 1%. Bone marrow analysis showed 50% of blasts expressed CD45, CD5, and cytoplasmic CD3, CD2 with only partial expression of CD7, CD11b, which was consistent with pre-T-cell ALL. Chromosomal breakage study confirmed Fanconi anemia.

The patient was treated for acute lymphoblastic leukemia (ALL) with induction chemotherapy at 75% dose reduction. His disease remained active as expected, but he developed severe liver impairment because of the extreme sensitivity and impaired tissue repair in Fanconi anemia patients. In spite of trials with repeated reduced doses of chemotherapy and steroids, the condition of the patient deteriorated and he eventually died because of active leukemia. This case demonstrates the rare form of transformation and progression of Fanconi anemia. Typically, Fanconi anemia patients progresses to myelodysplastic syndrome, which can then transform to acute myeloid leukemia.
This case also demonstrates the need for early diagnosis of Fanconi anemia and offering stem cell transplantation in order to avoid progression of the condition to such devastating outcome. These patients require chemotherapy dose adjustment in order to avoid organ toxicity.

Reference
Postpartum non-diarrhea-associated HUS is an unusual complication of pregnancy. It occurs as a single episode, either immediately or a few weeks after delivery. The disease is severe and is associated with microangiopathic hemolytic anemia (MAHA), thrombocytopenia, hypertension, and acute renal failure. It usually occurs in primigravida but has also been reported in multiparous women with a mean age of 27 years. Various triggering factors were implicated in causing postpartum HUS, especially pre-eclampsia, which was previously identified in 15% of patients with postpartum non-diarrhea-associated HUS.1

Early recognition is the key for successful treatment of this disease; however, the treatment of postpartum HUS remains controversial.

Case Report
A 22-year-old Saudi gravida 3, para 2 presented in labor at 39 weeks of gestation. She had no previous prenatal visits, and there was no history of bleeding, fever, or diarrhea. She had no medical history of hypertension. Physical examination was normal except for high blood pressure of 156/103 and petechiae of upper and lower limb.

The patient was diagnosed with mild preeclampsia and was found to have had intrauterine fetal death for which she underwent spontaneous normal vaginal delivery that revealed an abruptio placentae. The delivery was complicated by immediate vaginal bleeding, and she received 4 units of packed red blood cell as her hemoglobin level dropped to 60 g/dL from a base line of 87 g/dL.

Forty-eight hours after delivery, laboratory investigations revealed a leukocyte count of 14.9×10⁹/L; hemoglobin, 76 g/dL; hematocrit, 0.225, mean corpuscular volume (MCV), 70 fl, and random distribution of red cell width (RDW) 20%; a drop in platelet count to 36×10⁹/L from the base line of 161×10⁹/L was observed. The renal function had deteriorated over this period with creatinine level increasing from base line 55 µmol/L to 350 µmol/L within 24 hours. Other serum electrolyte levels were normal. Lactate dehydrogenase (LDH) at presentation was 662 U/L and had increased to 2756 U/L within 48 hours.

Peripheral blood smear showed a significant number of schistocytes with normal prothrombin time and activated partial thromboplastin time. Coombs test result was negative. Liver function test results showed a transient elevated alanine aminotransferase (ALT) level of 69 U/L while aspartate aminotransferase (AST) and indirect bilirubin levels were normal. Other laboratory investigations, including antinuclear antibody (ANA), anti-DNA, and complement C3 and C4 were normal.
C4 levels were within the normal limit. The results of antiphospholipid antibodies (APL) and lupus anticoagulants were negative. Hematology and nephrology teams were involved in the management and differential diagnosis, which revealed anemia secondary to bleeding along with coexisting iron-deficiency anemia; dilutional thrombocytopenia; preeclampsia; hemolysis, elevated liver enzyme, and low platelet (HELLP) syndrome; thrombotic thrombocytopenic purpura (TTP); and postpartum HUS.

The diagnosis of postpartum HUS was established with the exclusion of all the other differential diagnoses. Fresh frozen plasma (FFP), 15 mL/kg body weight of the patient, was given initially until plasma exchange (PE) started. PE was performed daily, and within 72 hours, the patient started responding as her hemoglobin count, platelet count as well as the LDH level improved.

After 11 sessions, her CBC and LDH normalized and her creatinine improved to a level of 99 umol/l and patient was discharged home on aspirin 81mg daily. After one month, she was seen in the outpatient clinic and she was in good health and her CBC, LDH, and creatinine levels were normal.

Postpartum Hemolytic Uremic Syndrome

literature reviews:

HUS and TTP are thrombotic microangiopathies (TMA) that are characterized by systemic and/or intrarenal aggregation of platelets, thrombocytopenia, and mechanical injury to erythrocytes (schistocyte). There is usually some degree of overlap in the clinical presentation of both syndromes with a prominent renal involvement in HUS and a central nervous system involvement in TTP.

HUS can be classified into diarrhea-associated HUS (D+HUS) and non-diarrhea-associated HUS (D−HUS).

The most common form of D+HUS is associated with infection by Escherichia coli and usually has an excellent prognosis in most cases, while D−HUS can be genetic, which is linked to mutation of gene factor H and/or gene membrane cofactor protein (MCP).

Other D−HUS presentation could be secondary to connective tissue diseases such as systemic lupus erythematous (SLE), scleroderma, malignant hypertension, radiation to the kidney, immunosuppressant therapy, pregnancy, and oral contraceptives. Usually, D−HUS has a worse prognosis compared to D+HUS, often resulting in uremia and/or death of the patient.

The association of HUS with pregnancy is rare, but a well documented event.

Postpartum HUS is an even rarer complication of pregnancy with unknown exact incidence but estimated to be around 1/25,000 pregnancies.

Robsen et al., first described postpartum HUS in 19686; and subsequently, different authors have reported few cases with similar presentation but different associated conditions, treatment approaches, and variable outcomes.

The reported cases and other relevant publications in the literature were reviewed and the salient findings are summarized in this manuscript. (Table 1).

The exact pathogenesis of postpartum HUS is unknown, but the disease can be triggered after an uncomplicated pregnancy as well as an abruptio placentae, spontaneous abortions, presence of circulating anticardiolipin antibody and lupus anticoagulant, and pregnancy-induced hypertension.

The association of postpartum HUS with the aforementioned conditions indicates that increased concentration of procoagulant factor and decreased fibrinolytic activity, loss of endothelial cell thrombomodulation, and decreased activity of von Willebrand factor-cleaving metalloprotease have major contributions to the development of postpartum HUS.

Postpartum HUS has also been associated with other deficiencies, including abnormalities of complement factor Hv1 and antibodies produced in response to verocytotoxin-producing E. coli O157:H7 infection.

In 1994, Sabai et al. postulated that the primary defect in postpartum HUS is an unidentified platelet-aggregating factor that causes deposition of microthrombin in the vessel wall causing occlusions in the microvasculature of the kidney, resulting in acute renal failure.

Recently, several reports have pointed out the relationship of thrombotic microangiopathic hemolytic anemia (TMHA) with the presence of APL.

SLE was the first autoimmune disease in which the association of TMHA with APL was recognized.

G. Espinosa reviewed 47 patients with TMHA associated with APL. HUS was the most common clinical presentation (26%), occurring postpartum period in 3 patients, followed by catastrophic antiphospholipid syndrome (APS) (23%), acute renal failure (15%), malignant hypertension (13%), TTP (13%), and HELLP syndrome (4%).

The diagnosis of postpartum HUS can be difficult, and it requires a very high index of suspicion. The presentation can be subtle and careful evaluation of CBC, peripheral blood smear, renal function in
### Table 1. Summary of the reported cases of post-partum hemolytic uremic syndrome (HUS).

<table>
<thead>
<tr>
<th>Author</th>
<th>No. of Patients</th>
<th>Onset after delivery</th>
<th>Clinical Presentation</th>
<th>Treatment</th>
<th>Response</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Segonds</td>
<td>3</td>
<td>10, 17, and 24 weeks</td>
<td>MAHA, thrombocytopenia, ARF, hypertension</td>
<td>Heparin + HD</td>
<td>1 complete recovery 1 developed Malignant hypertension and slight improvement in renal function after HD ESRD on H.D.</td>
<td>28</td>
</tr>
<tr>
<td>Kniaz</td>
<td>1</td>
<td>Immediate after spontaneous abortion</td>
<td>Severe preeclampsia, MAHA, ARF, thrombocytopenia</td>
<td>PE</td>
<td>Complete recovery</td>
<td>9</td>
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<tr>
<td>Pajor</td>
<td>1</td>
<td>Immediate after placental abruption (24 weeks of gestation)</td>
<td>ARF, MAHA, thrombocytopenia, fever and hypertension</td>
<td>FFP + HD</td>
<td>Complete recovery after 7 weeks</td>
<td>29</td>
</tr>
<tr>
<td>Jeng-Jong</td>
<td>1</td>
<td>Immediate after spontaneous abortion (15 weeks of gestation)</td>
<td>Typical picture of HUS</td>
<td>FFP + Heparin + HD</td>
<td>Partial recovery, ESRD on HD</td>
<td>8</td>
</tr>
<tr>
<td>Shemin</td>
<td>3</td>
<td>Postpartum</td>
<td>Typical picture of HUS</td>
<td>PE + Prednisone</td>
<td>Complete recovery</td>
<td>30</td>
</tr>
<tr>
<td>Takahashi</td>
<td>2</td>
<td>Postpartum</td>
<td>After successful cesarean delivery</td>
<td>FFP</td>
<td>1 complete recovery, 1 complicated by subarachnoid hemorrhage</td>
<td>31</td>
</tr>
<tr>
<td>Wu et al</td>
<td>1</td>
<td>After abruptio placent</td>
<td>Typical picture of HUS</td>
<td>PE</td>
<td>Complete recovery</td>
<td>20</td>
</tr>
<tr>
<td>Francisco</td>
<td>1</td>
<td>After emergency cesarean section due to abruptio placent</td>
<td>Typical picture of HUS and hypertension</td>
<td>FFP + HD + pulse methylprednisone</td>
<td>Complete recovery and persistent renal impairment</td>
<td>21</td>
</tr>
<tr>
<td>Lampinen K.</td>
<td>4</td>
<td>After emergency cesarean section</td>
<td>Typical picture of HUS and hypertension</td>
<td>2 PE only PE + HD + IV Ig + PE + Heparin + LWH + IV Ig + steroid</td>
<td>2 complete recovery after 2 weeks, complete recovery after 5 weeks, complete recovery after 4 weeks</td>
<td>32</td>
</tr>
<tr>
<td>Yu-Chieh</td>
<td>1</td>
<td>Under the indication of previous cesarean section</td>
<td>After the fourth episode of postpartum hemorrhage, full picture of HUS</td>
<td>PE + steroid + HD</td>
<td>Died</td>
<td>22</td>
</tr>
<tr>
<td>Mariarosaria</td>
<td>1</td>
<td>After cesarean section for twins</td>
<td>Full picture of HUS</td>
<td>PE</td>
<td>Complete recovery and persistent renal impairment</td>
<td>4</td>
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<tr>
<td>Al Sina</td>
<td>1</td>
<td>10 days postpartum</td>
<td>MAHA, thrombocytopenia, ARF and nephrotic syndrome</td>
<td>PE</td>
<td>Refractory hypertension and infectious complications</td>
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<td>Fakhari</td>
<td>15</td>
<td>Postpartum period</td>
<td>ARF, MAHA, thrombocytopenia</td>
<td>All PE PE + FFP PE + IV Ig</td>
<td>1 complete recovery 2 chronic renal impairment 12 patients with ESRD</td>
<td>34</td>
</tr>
</tbody>
</table>

MAHA - microangiopathic hemolytic anemia; ARF - acute renal failure; PE - plasma exchange; FFP - fresh frozen plasma; ESRD - end stage renal disease; HD - hemodialysis; LWH - low molecular weight heparin; IV Ig - intravenous immunoglobulin
POSTPARTUM HEMOLYTIC UREMIC SYNDROME

patients with abruptio placenta, and delayed post-partum hemorrhage should be considered. 22

Acute renal failure in late pregnancy in association with MAHA and thrombocytopenia were observed in 3 main disease entities, which include postpartum HUS, TTP, and severe pre-eclampsia with HELLP syndrome. 23

Patient with or possible APS and a repeated history of fetal loss are another group of patients where the diagnosis of postpartum HUS should be suspected.

The prognosis of postpartum HUS is generally poor. 24, 25

Most women died, or survived with severely impaired renal function, or progressed to end stage renal disease. One study showed that there was at least one serious long-term sequela in 78% of survivors.

The disease recurred in 50% of survivors. Fetal loss rate was as high as 80%; 10% of cases showed complete recovery of renal function.

However, early diagnosis and appropriate treatment, may reduce maternal mortality by 90%. 26

References


6. Robson JS, Martin AM, Rodgers VA, MacDonald MK. Irreversible postpartum renal failure: A new syndrome. QJM 1986;77:423


At present, PE is the most important treatment option for TTP/HUS that results in an improved outcome of 80–90%. 27

It is clear from the literature that most patients that recovered from postpartum HUS were treated with either plasma infusion along with hemodialysis or PE.

The role of steroid, heparin or other therapies remain unclear and should be considered only if the patient shows no response to PE.

Summary

Postpartum HUS is a rare but serious complication of pregnancy. It should be considered in certain complications such as abruptio placenta, fetal death, and APS. Careful monitoring of CBC in addition to other expressed features of the syndrome in the postpartum period will lead to early detection of HUS.

Immediate treatment with FFP and PE will improve maternal mortality outcome as seen in our patient.
case report


The goals of Comprehensive Care of Hemophilia (CCH) are to deliver the optimal care to patients with bleeding disorders and educate the patients and their families about the disease and how to minimize or prevent complications. Hemophilia is a rare bleeding disorder. Patients with hemophilia can develop many systemic complications. Although hematology service is the primary service for care of these patients, Hemophilia patients cannot be adequately treated in the settings of general hematology due to its complexity. The concept of multidisciplinary team care had changed the natural history of this disorder. This concept had been recognized by World Health Organization (WHO) and World Federation of Hemophilia (WFH).

There are many benefits expected from such program including:
1. Decrease mortality rate by receiving treatment through multidisciplinary team in Hemophilia Treatment Centre (HTC) by 70% compared to patient receiving treatment in general Hematology department.
2. Decrease hospitalization rate up to 40%.
3. Provide safe management of complicated cases such as seroconvert patients to HIV or Hepatitis C through contaminated blood products.
4. Multidisciplinary team can coordinate this management with better out come in (HTC) which minimize the need for excessive factor replacement during repeated bleeding episodes or procedures.

It is well known that more than 90% of the cost in hemophilia management is actual cost of factor so proper supervision and coordination in (HTC) can lead to optimal use of replacement therapy and decrease cost of treatment. Many studies had shown that organized and coordinated comprehensive care is less costly.

There are many approaches for establishment of Multidisciplinary team of CCH but most centers would have a comprehensive team that consists of: Pediatric Hematology, Adult Hematology, Physical Therapist, Social worker, Hemophilia Nurse Coordinator, Dentist, Orthopedic Surgeon, Infectious disease Pediatric Surgeon, laboratory technician, Psychologist and Genetic Counselor

The Role of Hemophilia Nurse Coordinator
Hemophilia nurse consider being the main person in the team and he/she plays many roles. He/she is the Resource person for patient & families, responsible for arrange CHC visits. He Receive & assess patient according to his/her current situation (emergency or regular appt.), insure that necessary & regular F/U blood tests for (Factor assay, virology, Inhibitor status) and X-rays results are ready with the patient with each visits. He/she communicates with patients before appointment and help patient with their referrals to other hospital services. The hemophilia nurse assists physicians in collecting data regarding various aspect of hemophilia and ensuring that the Registry is up-to-date, and continuo to be available for physicians, patient & families. Patient, families and community education is an important participation of the nurse in hemophilia care program. In program were home therapy is available, the nurse has to conduct regular home visits to e emphasis on the therapy and advice for changes in the home settings according to the needs of the patient. The role of the nurse in hemophilia care will always be evolving and will always be a challenge and be rewarding.

Reference
Charles Drew was born on June 3, 1904, and lived all his life in Washington, DC, travelling a lot for his career and education. Charles was one of those rare individuals who seemed to excel at everything he did and on every level and would go on to become a pioneer in the field of medicine. Charles’ early interests were in education, particularly, in medicine, but he was also an outstanding athlete. As a youngster, he was an award-winning swimmer and starred Dunbar High School in football, baseball, basketball, and track and field, winning the James E Walker Memorial medal as his school’s best all-around athlete.

In 1928, Charles decided to pursue his interest in medicine and enrolled at McGill University, Montreal, Canada. He was received as a member of the Medical Honorary Society and graduated in 1933 with Master of Surgery and Doctor of Medicine degrees, finishing second in his class of 127 students. He stayed in Montreal for a while as an intern at the Montreal General Hospital and at the Royal Victoria Hospital. In 1935, he returned to the United States, began working as an instructor of pathology at Howard University in Washington, DC, and was awarded the Rockefeller Foundation Research Fellowship.

Years back, while a student at McGill, he had saved a man by giving him a blood transfusion and had studied under Dr. John Beattie, an instructor of anatomy who was intensely interested in blood transfusions. Now, at Columbia, he wrote a dissertation on “Banked Blood” in which he described a technique he developed for the long-term preservation of blood plasma. Prior to his discovery, blood could not be stored for more than 2 days because of the rapid breakdown of red blood cells. Drew had discovered that by separating the plasma (the liquid part of blood) from the whole blood (in which the red blood cells exist) and then refrigerating them separately, they could be combined up to a week later for a blood transfusion. He also discovered that everyone has a certain type of blood (A, B, AB, or O) and thus are prevented from receiving a full blood transfusion from someone with different blood; everyone has the same type of plasma. Thus, in certain cases where a whole blood transfusion is not necessary, it was sufficient to give a plasma transfusion, which could be administered to anyone, regardless of their blood type. He convinced the Columbia University to establish a blood bank and soon was asked to go to England to help set up that country’s first blood bank.

Drew created a central location for the blood collection process where donors could go to give blood. He made sure all blood plasma was tested before it was shipped out. He ensured that only skilled personnel handled blood plasma to avoid the possibility of contamination. The Blood for Britain program operated successfully for 5 months, with total collections of almost 15,000 people donating blood and with over 5,500 vials of blood plasma. As a result, the Blood Transfusion Betterment Association applauded Drew for his work. Out of his work came the American Red Cross Blood Bank.

Charles Drew died on April 1, 1950, when the automobile he was driving went out of control and turned over. Drew suffered extensive, massive injuries, but contrary to popular legend, he was not denied a blood transfusion by an all-white hospital. He, indeed, received a transfusion but was beyond the help of the experienced physicians attending to him. His family later wrote letters to those physicians thanking them for the care they provided. Over the years, Drew has been considered one of the most honored and respected figures in the medical field and his development of the blood bank.

References

N.B. Due to scarcity of literature referring to Charles Drew’s biography, the websites are taken as references for this essay.
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REFERENCES
1. Summary of Product Characteristics NovoSeven®, Ed Feb 2004
Paroxysmal Nocturnal Hemoglobinuria (PNH): EASY TO MISS

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