thalassemia reports

PAGEPress Publications, Pavia, Italy I www.pagepress.org I

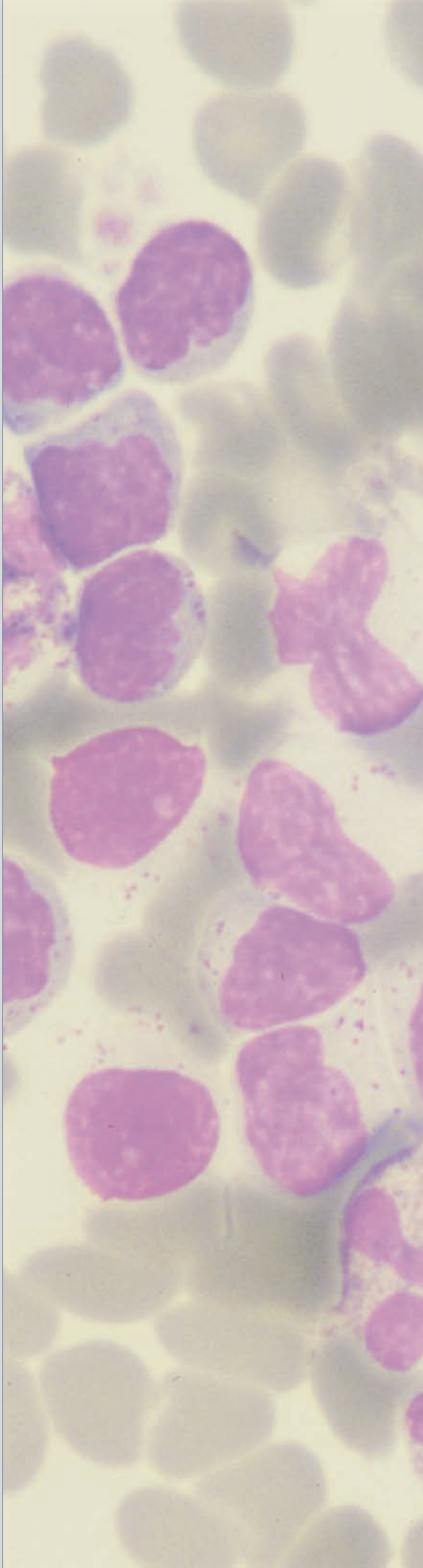
12th International Conference on Thalassemia and Hemoglobinopathies

11-14 May 2011, Antalya – Turkey

Guest Editors: Aurelio Maggio, Duran Canatan, Androulla Eleftheriou

THALASSEMIA REPORTS

ISSN 2039-4357 - eISSN 2039-4365



pagepress

THALASSEMIA REPORTS

is published by PAGEPress Publications, Italy.
The journal is completely free online at www.thalassemiareports.org
Publishing costs are offset by a publication fee charged to authors.

For more information
and manuscript submission:
www.thalassemiareports.org

Correspondence
Our publishing offices are located
in via Giuseppe Belli 4, 27100 Pavia, Italy.
Our telephone number is +39.0382.1751762
and our fax number is +39.0382.1750481.
E-mail: info@pagepress.org

All PAGEPress journals are Open Access.
PAGEPress articles are freely available online and deposited
in a public archive immediately upon publication.

12th International International Conference on Thalassemia and Hemoglobinopathies

11-14 May 2011

Antalya – Turkey

ORGANISED BY
Thalassaemia International Federation
Thalassaemia Federation of Turkey

WITH THE OFFICIAL SUPPORT OF
Governorship of Antalya
Municipality of Antalya

ORGANISING COMMITTEE

Duran Canatan
Chairman

Panos Englezos
Co-Chairman

Yurdanur Kılınç
General Secretary

Ramazan Uluhan
General Coordinator

Androulla Eleftheriou / Zeynep Karakaş
Scientific Coordinators

Duru Malyalı / Ahmet Varoğlu
Parents and Patients Coordinators

Rıfat Köse (MOH)
İrfan Şencan (MOH)
Sema Özbaş (MOH)
Serdar Mercan (MOH)
Gülsen Yüksel (TFT General Secretary)
Erman Oruc (TFT Treasurer)
Sefa Parlak (TFT Member)
Arzu Başaran (TFT Member)
Filiz Çıtacı (TFT Member)
Shobha Tuli (TIF Vice-President)
Riyad Elbard (TIF Treasurer)
George Constantinou (TIF Secretary)
Nailya Guliyeva (TIF Member)
Mikaela Panayiotou (TIF Office)

ADVISORY COMMITTEES

National Scientific Advisory Committee
Coordinator: Yurdanur Kılınç
Co-Coordinator: Zeynep Karakaş

Leyla Ağaoğlu
Kıymet Aksoy
Güçhan Alanoğlu
Sema Anak
Bülent Antmen
Hilmi Apak
Yusuf Ziya Aral
Yeşim Aydınok
Gönül Aydoğan
Nazlı Başak
Seher Başaran
Mahmut Bayık
Sinan Beksaç
Betül Biner
Şükrü Cin
Ümran Çalışkan
Mualla Çetin
Özgür Çoğulu
Akif Çürük
Muzaffer Demir
Buket Erer
Mehmet Ertem
Mesut Garipardıç
Hüseyin Gülen
Fatma Gümrük
Gülersu Irken
Semra Kahraman
İhsan Karadoğan
Hasan Kaya
Sabri Kemahlı
İbrahim Keser
Ahmet Koç
Erdal Kurtoğlu
Adalet Meral
Osman Özcebe
Mehmet Akif Özdemir
Ferda Özkınay
Gülyüz Öztürk
Alev Parlak
Türkan Patıroğlu
Fügen Pekün
Siret Ratip
Nazan Sarper
Murat Söker
Meral Sönmezoğlu
Melih Sözmen
Zafer Şallı
İlgen Şaşmaz
Naci Tiftik
Çetin Timur
İsmail Hakkı Timur
Bahattin Tunç
Zümrüt Uysal
Selma Ünal
Canan Vergin
Işın Yaprak
Sükrü Yenice
Mehmet Akif Yeşilipek
Yıldız Yıldırmak
İnci Yıldız
Mehmet Yılmaz

**all names are listed in alphabetical order by surname*

International Patients Advisory Committee
Chairpersons : Duru Malyalı (Turkey)
Loizos Perickleous (Cyprus)
Coordinator : Ahmet Varoğlu (Cyprus)

AUSTRALIA
Maria Kastoras

BANGLADESH
Kamrun Nahar

EGYPT
Mohammed M. El-Missiry
Manal Safwad Shoukry

GREECE
Varnavas Constantinou
Christina Stefanidou

GERMANY
Nicola De Nittis
Gabriella Giampaolo

INDIA
Rohan Anand
Anubha Taneja

IRAN
Mahmood Hadipour Dehshal

ITALY
Angelo Loris Brunetta
Tommasina Iorno

THE NETHERLANDS
Soroya Beacher
Elmas Çıtak

SAUDI ARABIA
Fouad S. Qaw

UAE
Saeed J. Al Awadhi

UK
Michael Michael
Chris Sotirelis

USA
Dawn Adler
Bob Ficarra

**all names are listed in alphabetical order by surname*

THALASSAEMIA INTERNATIONAL FEDERATION

12th International International Conference on Thalassemia and Hemoglobinopathies

11-14 May 2011

Antalya – Turkey

TABLE OF CONTENTS

THE INHERITED DISEASES OF HAEMOGLOBIN

Suthat Fucharoen

Thalassemia Research Center, Institute of Molecular Biosciences, Mahidol University, Nakornpathom, Thailand

Thalassemia is one of the common genetic disorders. Approximately 5-7% of the world's population is a carrier, and about 300,000 babies with severe diseases are born each year. β-thalassemia (β-thal) is frequent worldwide with low frequency. The α-thalassemia occurs across the tropical zone. Hb E is most concentrated in Southeast Asia. The complex gene-gene interaction leads to many thalassemic syndromes including such as homozygous β-thal, β-thal/Hb E and Hb Bart's hydrops fetalis. Management of thalassemia requires regular blood transfusion and effective iron chelation. In low-income countries it is not possible to give optimal blood transfusions to most patients. The iron chelator such as desferrioxamine is too expensive. Oral iron chelator is recently available, however, it is still too expensive or need special monitor. Stem cell transplantation is complex and costly. Sickle cell patients need primary care interventions including information and support for families, and care for patients with acute complications. Prevention of thalassemia is more important. This includes providing the best care to the patients and to prevent birth of new cases. Prevention strategies include community education, carrier detection, genetic counseling and prenatal diagnosis. Success in this program involves two major components, management and technology. WHO disease burden estimates are incomplete and speculative. The severe beta thalassemias probably account for 50,000 to 100,000 deaths per year, or 0.5-0.9% of all deaths of children under five in low- and middle-income countries. Each death accounts for 29.2 DALYs if it occurs before the child reaches the age of one. Thalassemia is causing an increasingly severe public health burden because it is very difficult to convince the governments that thalassemia is a public health problem compared with other communicable disease that these countries are handling. WHO report in 2004, recommend both North/South and local networks should be established as an aid to helping member countries to evolve these services. In conclusion, thalassemia is causing an increasingly severe public health burden for many countries in Africa, Asia and need proper program for the prevention and novel treatment.

PROMOTING ACCESS TO TREATMENT FOR PATIENTS WITH HAEMOGLOBIN DISORDERS

Perickleous Loizos

The presentation focuses on TIF's consistent mission and clear sense of direction in campaigning for preventive programs, since there is an apparent continuity in the lack of medical care for thalassemia patients in specific regions of the world. Hence, emphasis on screening programs have proved to be effective, particularly in countries in which medical care is weak. The presentation also delivers a summary of the history of the creation of TIF, and the crucial role of parents, patients, and medical practitioners who have been steadfast in their persistence on improving the lives of thalassemia patients. In addition, the presentation concentrates on personal reflections on the ways in which medical care and awareness has changed over time, and how the efforts of patients, parents and professional medical practitioners shaped the much-needed change. Emphasis is placed on the value of TIF and the need to sustain unity, which is vital to the success of the organization.

COMPONENTS OF AN EFFECTIVE LARGE SCALE PROGRAM FOR THE PREVENTION OF INHERITED HEMOGLOBIN DISORDERS; THE PARADIGM OF GREECE

Dimitris Loukopoulos

Biomedical Research Foundation; Academy of Athens, Greece

Thalassemia major or sickle cell disease result in a painful, miserable, and non-productive life of the patients along with a huge expense for their therapy, which can neither be optimal at all times nor easily met by the family and/or the national Health Authorities. Moreover, possibilities of cure by bone marrow transplantation are limited and the long awaited gene therapy is still an experimental exercise. Under these conditions, prevention remains as the only realistic solution to this date. Prevention, i.e., carrier identification and genetic counselling should be offered prospectively, i.e., before couples at risk proceed to pregnancy. In countries where the frequency of the abnormal genes is high, prospective prevention is a responsibility of the Health Authorities and should be provided to all prospective parents at the right time. Embarking on a large program of prevention starts with the commitment of the respective Authorities that they will totally support it; arguments which will support this step are the number of surviving patients and the expenses associated with their care along with the projection of these parameters over the years to come if no control measures are taken. Additional measures are the sensitization and ample information of the involved population (posters, leaflets, mass media and school), the organisation of laboratories which will offer carrier identification in a convenient and free of charge manner, and possibility of personal genetic counselling when both partners are diagnosed as carriers of the abnormal trait. Last but not least comes the possibility of prenatal diagnosis, which should be offered to all couples at risk in a descent and non-expensive manner, which will not only prevent birth of an affected child, but will also allow them to have healthy children. Large programs of this kind are now successfully running in several areas of the planet, where the incidence of hemoglobinopathies is high and should expand with no delay wherever a similar problem exists. The fact that preventing birth of an ever increasing number of new patients will ensure the use of the available resources for a better care of the patients actually surviving cannot be overemphasized.

THALASSEMIA PREVENTION PROGRAMME: A MODEL FROM IRAN

Ashraf Samavat

Head, Genetics Office, CDC, Ministry of Health & Medical Education, Iran

National Thalassemia prevention programme in Iran was started in 1997. It was planned after several years of advocacy by the haematologists involved and families affected by the disease. The plan focused on the pre-marriage screening covering 800,000 couples per year on average. Community and target groups' education, screening, genetic counseling, genetic diagnosis and epidemiologic surveillance have been the axial parts of the national plan. From the beginning the programme has been encountered questions and problems. Health authorities and managers of the programme to answer the questions planned different strategies and have been adopting tools and taking specific steps. Results of the different parts of the national programme proved continuous evolution and promotion concurrent with great achievements regarding the goals of the different strategies. The programme also paved the paths for the prevention and control of the other genetic diseases and proved for being a management model for Iran's comprehensive plan of community genetics. This article reviews and discusses main challenges and solutions to the problems and illustrates results to show the direct achievements of the thalassemia programme. The article also outlines the comprehensive community genetics of Iran to address the management model for the control of genetic diseases achieved by the integration of the thalassemia programme in the health system of the country.

HEMOGLOBINOPATHY PREVENTION PROGRAM IN TURKEY

Duran Canatan

Thalassemia Federation of Turkey, Antalya, Turkey

Thalassemia and abnormal hemoglobins are a serious health problem in Turkey. Very important steps for toward preventing thalassemia have been taken in Turkey by Ministry of Health (MOH), Turkish National Hemoglobinopathy Council (TNHC) and Thalassemia Federation of Turkey (TFT) since 2000. In 1993, a law was issued called *Fight Against Hereditary Blood Disease especially for thalassemia and hemoglobinopathies*. The law commends to prevent hemoglobinopathies and to treat all patients with hemoglobinopathy and thalassemia. A pilot project was started and centers were created in the MOH Hospitals in the southern provinces of Turkey. In 2000, TNHC was installed to combine all centres, foundations, and associations into one organization controlled by the MOH. In 2001, the MOH and the TNHC made an inventory of all recorded patients with thalassemia and abnormal hemoglobins in Turkey, registering at least 4513 patients. In 2002, writen regulations for the Fight Against Hereditary Blood Disease were published. MOH and TNHC selected 33 cities situated in the Thrace, Marmara, Agean, Mediterranean and South Eastern regions with high birth prevalence of severe hemoglobinopathies. In 2003, the hemoglobinopathy scientific committee was set up, a guidebook was published and a national Hemoglobinopaty Prevention Program (HPP) was started in these high risk cities on 8th of May. This program has been run in these cities successfully.In 2005, TFT was established as a secular society organization instead of TNHC. In 2007, National Thalassemia Prevention Campaign (NTPC) was organized for public education by TFT. This campaign contributed very important supporting to HPP in Turkey, because totally 62,682 people such as health workers, students, teachers, demarches, religion officers and the other many people were educated for preventing thalassemia and hemoglobinopathies. In 2009, National Thalassemia Education Seminars (NTES) for health personels have been planned in 26 cities by MOH and TF. A total 3600 health persons were educated on thalassemia preventing and therapy with NTES in 18 centers in 2009 and 2010. In conclusion, according to reports of MOH, 46 first level hemoglobinopathy diagnosis centers, 5 second level diagnosis and therapy center and 5 third level prenatal diagnosis center were set up and licensed in 30 cities between 2003 and 2009. While premarital screening tests were 30% of all couples in 2003, it was increased continuously during 6 years and it was reached 81% in 2008. The number of newborns with thalassemia and hemoglobinopathies was 272 in 2002, it was decreased to 23 in 2008, as a result there has been a 90% reduction in new affected births.

Key words: hemoglobinopathy prevention program, Turkey.

PREIMPLANTATION GENETIC DISORDERS IN THE PREVENTION OF THE HAEMOGLOBIN DISORDERS

Semra Kahraman

Istanbul Memorial Hospital ART and Genetics Center, Turkey

Preimplantation genetic diagnosis (PGD) is currently an alternative for couples with high risk of pregnancies with genetic anomalies. PGD offers to these couples the possibility of avoiding the need to terminate affected pregnancies, since it allows the selection of unaffected embryos for transfer. PGD for inherited disorders has become extremely accurate (99.5%), and may currently be performed for any genetic condition even available haplotypes. PGD has been performed for more than 100 different conditions resulting in the birth of at least 1000 healthy children free of genetic disorder. PGD is presently also used together with preimplantation HLA typing for treatment of affected sibling with genetic and acquired disorders requiring HLA matched stem cell transplantation. This is not only to allow couples to have an unaffected child but also to select a potential donor progeny for stem cell transplantation. It is therefore of a great value for hematopoietic and other life threatening diseases as stem cells in the cord blood and bone marrow from an HLA compatible newborn can be used for transplantation without graft rejection, thus saving an affected children's life. PGD for HLA matching has also been used as a primary indication in cases not requiring mutation testing Haemoglobin

disorders can be effectively reduced through a strategic balance of these management and prevention programs and PGD is an extremely useful tool for prevention (i.e. leukemia) (*Verlinsky and Kuliev, 2003*). Considering that the theoretical probability of finding HLA compatible and mutation free embryos is about 18%, obtaining a sufficient number of suitable embryos as well as good quality necessitates a higher number of oocytes to be fertilized and embryos to be biopsied for a given cycle. Due to a small number of children per family, only one third of patients are able to find an HLA identical sibling. This may further be improved by 3% using an extended family research for a matched related donor. In the remaining patients the only resort is the identification of a matched unrelated donor, which may be maximized by establishing national registries. Apart from being a valuable treatment approach, there may exist several patients or cycle-specific limitations and it seems that not all couples can benefit from the present procedures. In Turkey, thalassemia is the most commonly seen genetic disorder the rate of thalassemia carriers is about 3-4%. The majority of our PGD cases are thalassemia carriers. They do not only require thalassemia mutation analysis but also HLA typing for their affected child. PGD results of 236 Turkish couples with or without HLA typing will be presented and discussed.

THE MOLECULAR BASIS OF β-THALASSEMIA IN TURKEY

A. Nazli Basak

Bogazici University, Dept. Molecular Biology and Genetics, Director, Neurodegeneration Research Laboratory, Istanbul, Turkey

As in many other Mediterranean countries, β-thalassemia is a major public health concern in Turkey. The gene frequency is estimated to be 2.1% throughout the country, and in certain regions this number increases to 10%. The number of β-thalassemia carriers is approximately 1.300.000, and the number of β-thalassemia patients is around 4000 in Turkey. The number of affected births is higher than expected, because the birthrate is still high in Turkey and the number of consanguineous marriages is above 60% in the eastern parts of the country. In contrast to most other Mediterranean countries, β-thalassemia in Turkey is very heterogeneous at clinical level and the transfusion-dependent severe β-thalassemia is the major predominating form. Today we know that the clinical heterogeneity is largely the result of the molecular heterogeneity, which can be explained by the unique geographical location and rich historical background of the country, which forms a bridge between Europe and Near East. Historically, Turkey served as an important crossroad among civilizations, cultures and continents for several centuries. The molecular analysis of β-thalassemia was initiated for the first time in Turkey in our laboratory in 1987; at that time the molecular basis of β-thalassemia was largely unknown in Turkey. The main purpose was to establish a comprehensive prenatal diagnosis strategy, based on DNA analysis, similar to successful examples in many at-risk populations. Between 1987 and 2008, more than 3100 chromosomes from β-thalassemia patients and families were investigated at our Center. As in most other eastern Mediterranean countries, by far the predominating mutation in Turkey is the IVS-1-110 (G-A) lesion, followed by the relatively mild IVS-1-6 (T-C) mutation. The ratio of β^0 to β^+ mutations is 1:1, but since the majority of β-thalassemia cases bears the severe IVS-1-110 mutation, most of these mutations give rise to β-thalassemia major in homozygous or compound heterozygous combinations. The six most common mutations in Turkey add up to 70%, and the overall frequency of the first 12 mutations, including HbS is 83.5%. Through examination of the collective results of over 3100 β-thalassemia chromosomes and their origins in Turkey, we established the distribution pattern of β-thalassemia and HbS alleles in Turkey and constructed a nationwide distribution map of β-thalassemia mutations, which facilitated a more focused molecular genetic approach to prevention. I will demonstrate in my presentation that despite the high degree of molecular heterogeneity, the above approach, combined with the advent of PCR-based techniques and improved methodologies of early fetal sampling have made heterozygote screening and prenatal diagnosis of β-thalassemia feasible in Turkey.

THE CURRENT STATUS OF GENE THERAPY OF THE β-THALASSEMIA SYNDROMES

George Stamatoyannopoulos

University of Washington, Seattle, WA, USA

Attempts to develop a gene therapy for the β thalassemia syndromes were initiated about 25 years ago. Following several years of disappointments, the very difficult task of developing stable viral vectors that allow high level of globin gene expression was finally successful and the first patient with severe HbE/β° thalassemia was treated with a β-globin gene vector in 2008. In this talk I will review the current experience with the gene therapy of β thalassemia and I will discuss developments expected to take place in the near future.

FROM GENOTYPE TO PHENOTYPE IN THALASSEMIA

Renzo Galanello

Pediatric Clinic 2 and Thalassemia Unit, Ospedale Microcitemico, University of Cagliari-ASL8, Italy

The clinical manifestations of β-thalassemia are extremely heterogeneous, ranging from severe transfusion-dependent anemia, to the mild non transfusion dependent thalassemia intermedia and to the asymptomatic carrier state. The remarkable phenotypic variability is primary due to variations in the different globin genes (primary gene modifiers). The main pathophysiological determinant of the severity of β-thalassemia syndromes is the extent of α/non-α globin chain imbalance. Therefore, any factor capable of reducing the globin chain imbalance may have an ameliorating effect on the clinical picture. The most common mechanisms responsible of the amelioration of the phenotype are mild or silent β thalassemia alleles, coinheritance of α thalassemia, or of genetic determi-

nants associated with increased γ globin chain production. Rarely, other complex mechanisms including dominantly inherited β thalassemia, somatic deletion of β globin gene and coinheritance of extra α globin genes with heterozygous β thalassemia have been reported. In addition to the variability of the phenotype resulting from primary gene modifiers, other genetic factors (secondary gene modifiers), mapping outside the β and α globin cluster, may influence the disease complications. Among these factors the ones best so far defined are those affecting bilirubin, iron and bone metabolism. However, the new methods of DNA analysis (i.e. GWAS and related methods) are expect expand the number of genes or gene variants involved in the phenotypic variability and in the response to treatment of β thalassemia.

GAMMA GLOBIN GENE REGULATION

Swee Lay Thein

King's College London/King's College Hospital, James Black Centre, London, UK

Our understanding of haemoglobin control, including the persistence of fetal haemoglobin (HbF, $\alpha_2\gamma_2$) synthesis in adults, is historically based on Mendelian models of inheritance of natural mutants. Indeed, a series of mutations at the *HBB* cluster that impairs the switch from fetal to adult haemoglobin have been characterised in a syndrome termed hereditary persistence of fetal haemoglobin (HPFH). These Mendelian forms of HPFH are caused by two classes of mutations: deletions removing substantial regions of the β globin gene cluster, and point mutations in the promoter of the γ globin genes (*HBG1* and *HBG2*). The molecular mechanisms for the persistent γ globin gene expression has been ascribed to altered binding to *trans*-acting factors at the site of the point mutations. Deletional HPFH is associated with both loss of repressive intergenic sequences and juxtaposition of the 3' enhancer sequencer sequences into close proximity with globin genes. Although the increases in HbF mirror the molecular diversity of the HPFH mutations, within each molecular class, a range of HbF levels following a continuous distribution and that do not fit clear Mendelian inheritance models have been observed. Variable increases in HPFH levels have also been noted in individuals with sickle cell anaemia (SCA, HbSS) and β thalassemia. While some of the variability may be related to variation at the *HBB locus*, discordant HbF levels among sibships with identical mutant β alleles suggest the influence of *trans*-acting factors. It is now clear that the variable HbF levels among individuals with identical HPFH mutations and in disease occurs on a background of common HbF variation observed among healthy adults that persists as a quantitative gene trait. Positional cloning has led to the identification of a quantitative trait locus (QTL) on 6q (*HBS1L-MYB* intergenic polymorphism, *HMIP*) in an Asian Indian family, and more recently a rare variant in KLF1 on chromosome 19p in a Maltese family with HPFH. Recent genome-wide association studies (GWAS) not only 'rediscovered' the known loci – *Xmn1 HBG2*, and HBS1L-MYB intergenic region (*HMIP*) on chromosome 6q23 – but identified *BCL11A* on chromosome 2p16, previously known as an oncogene. *BCL11A, HMIP* and the *HBB* QTL account for 20-50% of the phenotypic variation in HbF levels, both in healthy adults and in patients with a significant impact on clinical severity of the β haemoglobinopathies. Two plausible mechanisms have been suggested for the biological effect of the QTLs on HbF expression: 1) direct effect on γ globin gene expression (activation or repression of HBG transcription) and 2) alteration of the kinetics of erythroid maturation mimicking a situation encountered in stress erythropoiesis that results in the accelerated expansion of the early erythroid progenitors that still retain a predominantly γ globin expression programme. In this respect both *BCL11A* and *HMIP* have also been shown to have a pleiotropic effect on other haematological parameters. Functional and transgenic mice studies have shown that *BCL11A* acts as a repressor of γ globin expression. *BCL11A* exerts its effect through association with various proteins – SOX6, GATA1 and the repressive nucleosome remodelling and deacetylase complex (NuRD). As for *HMIP*, the interval has all the makings of a distal regulatory locus and it is suggested that these regulatory elements distally control the flanking genes – HBS1L and MYB that indirectly influence HbF expression and evidence so far suggests that MYB is the culprit. As the story unfolds, KLF1 (also known as EKLF) has reemerged as a key erythroid regulator. KLF1 binds to the *BCL11A* promoter. In the light of recent findings, KLF1 may mediate the switch through a dual mechanism – activation of β globin gene and repression of γ globin gene by *trans*-activation of *BCL11A*. In a final twist, a recent study showed that knockdown of *MYB* in human erythroid progenitors was associated with decreased *KLF1* expression. Ultimately, it may be that *MYB* is the master regulator – reduction of *MYB* accelerates erythroid differentiation with expansion of the F cell pool; at the same time, with downregulation of *KLF1, BCL11*A remains repressed with persistence of γ globin expression. Chromatin-modifying factors may also derepress the globin gene by altering their methylation status and these include the arginine methyltranferase 1 (PRMT1) and FoP (friend of PRMT1). Challenges that remain include delineating the causal variants in these HbF QTLs and identifying other loci in the molecular network to improve the prediction of one's ability to produce HbF. Nonetheless, recent discoveries have already provided novel targets for reactivating the γ globin gene in the treatment of the β haemoglobin disorders.

FETAL GLOBIN GENE INDUCTION: CLINICAL STAGE ORAL AGENTS FOR TARGETED THERAPEUTIC REGIMENS

Susan P. Perrine

Boston University School of Medicine & Phoenicia BioSciences, Boston, MA, USA

β thalassemia intermedias are serious anemias worldwide, in which widespread organ damage occurs due to hemolysis from globin chain imbalance, bone marrow

expansion, and excessive gastro-intestinal iron uptake, and eventually progresses to transfusion-dependency. Pharmacologic augmentation of fetal globin expression by even small increments can reduce the globin chain imbalance and hemolytic anemia, and has shown proof-of-concept in eliminating transfusion dependency. Combining oral pharmacologic HbF-inducers with differing mechanisms of action offers a feasible approach for high-level fetal globin induction. Our group has employed molecular modeling and high throughput screening to interrogate chemical libraries for fetal globin-inducing compounds, and has identified epigenetic modifiers, specific inducers of the fetal globin gene promoter, which displace repressor complexes, and agents that suppress the repressor Bcl-11A. These newly identified therapeutics include a panel of therapeutics already FDA-approved for other medical conditions. Multiple newly identified therapeutics have 10 to 100-fold higher potency in inducing fetal globin mRNA than prior-generation agents. Evaluating the activity of different drugs on erythroid progenitors cultured from diverse thalassemia genotypes and genetic modifiers (QTLs) can guide personalized regimens. These clinical-stage therapeutics now make fetal globin induction a feasible treatment approach for worldwide use.

NEW ADVANCES IN BLOOD SAFETY: A REGIONAL EXPERIENCE IN FRANCE OF PATHOGEN INACTIVATION TREATMENT OF LABILE BLOOD COMPONENTS TO PREVENT TRANSFUSION–TRANSMITTED INFECTIONS

Jean Pierre Cazenave

Université de Strasbourg, France

Transfusion of labile blood components (LBC) is vital for patients in the absence of alternative treatment. Transfusion transmitted infections by LBC and blood derived drugs have always been feared by patients and doctors. EFS Alsace provides all LBC to transfuse 2 million inhabitants. Since 2006, we implemented universal pathogen inactivation (PI) treatment of platelet (PI-PC) and plasma (PI-FFP) with amotosalen and UVA light [INTERCEPT Blood System™ (IBS)]. As part of a mandatory national hemovigilance (HV) program, we prospectively collects >99.5% data on production, distribution, and response to transfusion of all LBC. For PC, data collection covered three periods: 1) PC in plasma, 2) PC with platelet additive solution, and 3) PC prepared with PI. Data on component utilization were analyzed for all patients receiving PC in each period and for the subset of hematology-oncology patients to evaluate PC use by an intensely transfused population. About 2000 patients received PC in each period. Platelet and RBC use per patient was not increased after PI and the incidence of acute transfusion reactions (ATRs) was significantly reduced ($P<0.02$). In addition, since routine use of more than 77,000 PI-PC there have been no cases of transfusion-related sepsis (TRS). In contrast, TRS continues to be reported in EFS regions not using PI-PC. EFS Alsace led a multi-national HV program to evaluate the safety of PI-FFP after each transfusion. The primary outcome was the incidence of ATRs within 24 h of transfusion. As of 2009, 3232 patients (2884 adults, 160 children, and 188 infants) with a primary indication for plasma transfusion due to a hematology disorder (23.1%), surgery (32.5%) or a general medical condition (44.4%) received 7483 transfusions (19,069 PI-FFP components). The study included patients undergoing liver transplant and therapeutic plasma exchange. ATRs were reported for 8/7483 transfusions (0.11%) and 8/3232 patients (0.25%). Five ATRs were of Grade-1 severity and 3 classified as serious. No deaths or episodes of TRALI attributed to a PI-FFP were reported. EFS Alsace has introduced developmental studies with the IBS for RBC using S-303 to initiate Phase 3 clinical trials in 2011. Universal implementation of PI of LBC is a major step to improve safety against infection in transfusion. Routine implementation of PI technology for platelet and plasma components in a French regional transfusion centre demonstrated feasibility, efficacy, and safety.

BLOOD SAFETY ACCORDING TO EUROPEAN AND INTERNATIONAL STANDARDS

Mahmut Bayik

President of Blood Banks and Transfusion Society of Turkey (BBTST)

Blood safety depends on: The recruitment and retention of blood donors who are at low risk of transmitting infection, safe blood collection procedures, correct testing for transfusion-transmissible infections, blood grouping and compatibility testing and the appropriate use and safe administration of blood. *WHO strategy for blood safety.* A well-organized, nationally-coordinated blood transfusion service that can provide adequate and timely supplies of safe blood for all patients in need, the collection of blood only from voluntary non-remunerated blood donors from low-risk populations, testing of all donated blood for transfusion-transmissible infections, blood grouping and compatibility testing, the appropriate clinical use of blood, including the use of alternatives to transfusion wherever possible, and the safe administration of blood and blood products, quality system covering all stages of the transfusion process. *Requirements for safe blood policy*. National blood policy and plan, legislation and regulation, well-structured blood transfusion service (BTS), specific budget allocation, and standards for blood transfusion services. *An effective national quality system requires*. National quality policy and plan, quality officers at national and local levels, quality standards, documentation system (with special software for haemovigilance and traceability) (RFID system), training of all staff, assessment of the quality system. *Requirements for safe blood supply.* National blood donor programme, identification of low-risk donor populations, national criteria for donor selection, safe blood collection procedures, donor notification and referral for counseling, donor records. Requirements for quality in testing, processing, storing and transportation of blood: National strategy for TTI testing and blood grouping, evaluation and reliable supply of test kits and reagents, sustainable programme that responds to clinical demands, application of good manufacturing practice, specialized storage and transportation equipment, regular monitoring and maintenance of equipment. *The appropriate clinical use of blood requires.* National policy and guidelines on transfusion, training of all staff involved in transfusion, availability of alternatives to transfusion, hospital transfusion committees, blood request form, blood ordering schedule, system for monitoring transfusion practice. *Prevention from transfusion reactions requires.* standard operating procedures for bedside transfusion, training in bedside transfusion, haemovigilance system for monitoring, reporting and investigating adverse events associated with transfusion *European Council regulations.* The 2002 Directive of the European Parliament and of the Council (2002/98/EC), setting standards of quality and safety for the collection, testing, processing, storage and distribution of human blood and blood components and amending Directive 2001/83/EC. *The Council of Europe Guide to the preparation, use and quality assurance of blood components.*

BLOOD TRANSFUSION THERAPY IN THALASSAEMIA MAJOR

John B. Porter

University College London, UK

Blood transfusion therapy in thalassemia major aims to deliver the optimal amount of blood in the safest manner.[1,2] Strategies for minimising risks from allo-iminisation, other transfusion reactions, and infection risks have been detailed in the Thalassemia International Federation (TIF) guidelines. Alloimunisation can be minimized with careful donor selection and screening; voluntary, regular non-remunerated blood donation is the recommended standard. Confirmation of the genetic diagnosis of thalassemia major is important before regular transfusion begins. Before the first transfusion, extended red cell antigen typing of patients for C, E and Kell as a minimum are commended. At each transfusion, ABO, Rh(D) compatible blood should be given with additional matching for C, E and Kell antigen and with, full cross-match and screen for new antibodies before each transfusion. A long-term record of red cell antibodies, transfusion reactions and annual transfusion requirements for each patient is important and particularly useful when patients move their place of treatment. Leuco-reduced packed red cells with pre-storage filtration is recommended, but blood bank pre-transfusion or bedside filtrations are acceptable alternatives. Washed red cells are only indicated for patients who have severe allergic reactions. Use red cells stored in CPD-A, as fresh as possible (less than one week old) and in additive solutions for less than 2 weeks is optimal. Transfusion should occur every 2-5 weeks, maintaining pre-transfusion Hb above 9-10.5 g/dL, but higher levels (11-12 g/dL) may be necessary for patients with heart complications. The post-transfusion Hb should not be not higher than 14-15 g/dL. Future developments including pre-treatment of the red cell product to reduce infection risks including prior related diseases will be discussed.[3] The rationale for guidelines about the transfusion regime that optimises the balance between over and under-transfusing patients will be discussed.[4,5] This has been determined only in an Italian population of thalassemia patients and it is likely that this optimal rate may vary with the underlying genotype of thalassemia being treated. For example in E-β thalassemia, the optimal rate may be significantly less. Because of changes in the preparation of red cell products in recent years, there is variability in iron content of a 'unit' of blood. It is recommended that each centre involved in the treatment of thalassemia know the average haematocrit and volume of the blood product that they are using, as this impacts on response to chelation therapy. Based on the rates of blood transfusion, recommendations about maintenance dosing with chelation regimes involving deferasirox or desferrioxamine can now be given.[6]

References

1. TIF. Guidelines for Clinical Management of Thalassaemia. (Book). Edited by: Capellini, N, Cohen, A, Eleftheriou, A, Piga, A, Porter, JB, Taher, A: Thalassaemia International Federation, Nicosia, Cyprus, 2008, Revised 2nd ed.
2. Porter J. Blood transfusion: quality and safety issues in thalassemia, basic requirements and new trends. Hemoglobin. 2009 33 Suppl 1. S28-36.

3. Solheim BG. Pathogen reduction of blood components. Transfus Apher Sci 2008;39:75-82.
4. Cazzola M, De Stefano P, Ponchio L, et al. Relationship between transfusion regimen and suppression of erythropoiesis in beta-thalassaemia major. Br J Haematol 1995;89: 473-8.
5. Cazzola M, Borgna-Pignatti C, Locatelli F, Ponchio L, Beguin Y, De Stefano P. A moderate transfusion regimen may reduce iron loading in beta- thalassemia major without producing excessive expansion of erythropoiesis. Transfusion 1997;37:135-40.
6. Cohen AR, Glimm E, Porter JB. Effect of transfusional iron intake on response to chelation therapy in beta-thalassemia major. Blood 2008;111:583-7.

OVERVIEW OF THE CURRENT ISSUES AND ADVANCES IN HAEMOPOIETIC STEM CELL TRANSPLANTATION FOR β-THALASSEMIA MAJOR

Sema Anak

Dept. of Pediatric Hematology/Oncology, Istanbul School of Medicine, Istanbul University, Turkey

β-thalassemia major is a genetic defect that causes an ineffective erythropoiesis with hemolysis. The main treatment principles are regular red blood cell (RBC) transfusions with iron chelation therapy for transfusion-related iron overload and supportive care. Bone marrow transplantation (BMT) is the only possible curative treatment for β thalassemia major. Turkey is one of the countries where the prevalence of β thalassemia major is still very high and causes an important burden for health facilities. Yesilipek *et al.* recently presented the results of HSCT for β thalassemia patients on behalf of Turkish Pediatric Stem Cell Transplantation Group. Between Jan 1991-June 2009, 245 children with β thalassemia major underwent first allo HSCT in 9 centers in Turkey. M/F ratio was 129/116 and the median age was 6.6 (range 1-22 years). Forty-one patients were in Class I, 137 in Class II, 63 in Class III and class is not known in 11 patients. Stem cell sources were bone marrow in 88, peripheral blood in 137 and cord blood in 20. All donors were HLA matched related donors. Conditioning regimens consisted of BU + CY in 95, BU+CY+ATG in 100, Pesaro Protocol 26 in 40, BU+CY+ Tio-Thepa (TT) in 3. CsA alone or in combination with MTX or metylprednisolon is used for graft versus host disease (GvHD) prophylaxis. Median follow-up period after HSCT was 61 months, (14-231 months). Acute GvHD was observed in 42 children, 31 of which had Grade II-IV. Chronic GvHD have occurred in 29 patients, 8 with extended form. Thalassemic reconstitution has been observed in 43 transplantations. Nineteen patients expired in the first 100 days and transplantation related mortality (TRM) was 7.75%. EFS (thalassemia free and alive) and OAS were 68.0% and 85.0%, respectively. In conclusion, the role of transplantation for β-thalassemia major, which is a chronic, nonmalignant condition, depends on the available supportive care measures in that country. Still, with best conditions, most persons die of thalassemia or of complications related to its treatment in the fourth or fifth decades of life. So, the successful transplantation outcomes reported should encourage the staff and families for the possibility of HLA-matched sibling BMT, when there are limited resources.

TRANSPLANTATION FOR THALASSEMIA IN LOWER-RESOURCE SETTINGS

Lawrence Faulkner,[1] Naila Yaqub,[2] Sadaf Khalid,[1] Tatheer Zhara,[1] Saqib Ansari,[3] Tasneem Farzana,[3] Tahir Shamsi[3]

[1]Cure2Children Foundation, Florence, Italy; [2]Pakistan Institute of Medical Sciences Children's Hospital, Islamabad, Pakistan; [3]National Institute of Blood Diseases, Karachi, Pakistan

Thalassemia major (TM) is the most common deadly genetic disorder, a major cause of chronic non-infectious morbidity and financial burden in many low- and middle-income regions. In these settings few children reach adulthood because proper long-term supportive care in seldom available. Bone marrow transplantation (BMT) is the only available curative modality and it can be very successful and cost-effective for young children with low-risk features and a compatible related donor. However, in countries where TM is most prevalent there is a dire shortage of BMT centers. The Cure2Children Foundation has supported a feasibility study evaluating safety, efficacy and costs of developing a new BMT center in an underserved lower-middle-income country with relatively untrained professionals within a structured collaboration and knowledge-transfer program. A total of 24 patients who underwent BMT in Pakistan between September 2008 and August 2010 were included in this prospective analysis, 17 from an established bone marrow transplant center, the National Institute for Blood Disease in Karachi, Pakistan and the initial 7 BMTs from a start up unit in a government civil hospital, the Pakistan Institute of Medical Sciences Children's Hospital in Islamabad. Patients were matched for age, nutritional status, growth, disease, diseases status and post-BMT follow-up time. All patients were younger than 10 years at BMT, received the same conditioning regimen and all needy families could rely on a support program throughout the 8-month post-transplant period. The Cure2Children Foundation provided professional and financial support as well as a structured web-based data management and cooperation platform. At a median follow up of 19.6 months (range 8.7 to 31.5) actuarial thalassemia-free survival is 85.6% and 85.7% and overall survival 94.1% and 85.7% in the established and start-up center respectively with no statistically significant differences. Other outcome indices like Infectious complications, engraftment parameters, transplant-related complications, and post-BMT performance scores did not differ. The median cost of matched-related transplants in the start-up center, including pre-BMT evaluation, was 11,513 USD (range 7518 to 21,176). Within structured cooperation strategies bone marrow transplantation for thalassemia major can be performed safely, effectively, and affordably even in start-up centers in lower-middle-income countries, like Pakistan, where most thalassemia patients live. This observation may have important implications to increase access to cure for thalassemia world-wide.

PATHOPHYSIOLOGY OF IRON OVERLOAD IN THALASSAEMIA SYNDROMES

John B. Porter

University College London, UK

Excess iron from blood transfusions and/or from elevated dietary absorption accumulates as tissue storage iron as ferritin and haemosiderin. Increased iron absorption is driven by suppression of hepcidin synthesis in the liver by ineffective erythropoiesis (IE), which can be detected indirectly by raised plasma levels of GDF15 and circulating transferrin receptors. This rate is small however compared with the rate of iron accumulation from transfusion. This depends on the patient's underlying disease; 0.3 and 0.5 mg/kg/day in thalassemia major, although higher or lower rates are seen in a small proportion of thalassemia patients.[1] Transfused red cells are catabolized in macrophages, releasing iron that saturates transferrin, forming plasma non-transferrin bound iron (NTBI). The uptake of plasma NTBI into tissues occurs by a different mechanism and in different tissues than with transferrin-mediated iron uptake, leading to excess accumulation in the liver, heart, pancreas, anterior pituitary, thyroid, and parathyroid glands. NTBI is also increased by IE, so that a simple relationship between high NTBI levels and iron stores are not always seen. Furthermore, patients with thalassemia intermedia often have high NTBI and LPI levels but rarely accumulate extra-hepatic iron unless they commence regular transfusion.While storage iron is not directly toxic, this contributes to increasing the magnitude of labile intracellular iron. Plasma or intracellular labile iron pools can be toxic because these iron the formation of free radicals that cause oxidative damage to lipids, proteins, and DNA. Injury to these tissues may result in liver fibrosis and cirrhosis, hypogonadodrophic-hypogonadism, hypothyroidism, hypoparathyroidism and cardiomyopathy. The highest concentration of tissue iron is found in the liver, and this concentration (LIC) reflects body iron loading in a predictable way.[2] Overall iron burden (most accurately assessed by liver iron measurement) correlates with clinical outcome. Iron burden is reflecred by serum ferritin levels, the long-term control of which correlates with long-term survival. However single measures of ferritin or LIC do not predict myocardial iron concentration, as measured by T2*. Some patients are remarkably resistant to extra-hepatic iron accumulation despite very high LIC values, while others appear to develop myocardial iron loading despite good control of ferritin or LIC. The reasons for this variability are currently under investigation, but the underlying haemoglobinapathy,[3] the rate of transfusion, the modality of chelation and as yet undefined genetic polymorphisms are likely to contribute to this variability.

References

1. Cohen AR, Glimm E, Porter JB. Effect of transfusional iron intake on response to chelation therapy in -thalassemia major. Blood 2008;111:583-7.
2. Porter JB and Shah FT Iron overload in Thalassaemia and Related conditions: Therapeutic Goals and Assessment of Response to Chelation therapies. Haem Onc Clinics of North America, 2010, 24, 6, 1109-1130.
3. Porter JB. Pathophysiology of transfusional iron overload: contrasting patterns in thalassemia major and sickle cell disease. Hemoglobin. 2009;33 Suppl 1:S37-45.

OVERVIEW OF CURRENT CHELATION PRACTICES

Yesim Aydinok

Ege University, Department of Pediatric Hematology, Izmir, Turkey

Deferoxamine (DFO) is reference standard therapy for transfusional iron overload since 1980s. Although it is a highly effective iron chelator, compliance problem to subcutaneous administration of DFO remained as the major problem. The oral chelator Deferiprone (DFP) has not marketing in North America, however, has been licensed in India since 1994 and the European Union (EU) granted marketing approval for DFP in 1999. DFP is approved specifically for patients with thalassemia major when DFO is inadequate, intolerable or unacceptable. There are still limited data available on the use of DFP in children between 6 and 10 years of age, and no data on DFP use in children under 6 years of age. Subsequent oral chelator Deferasirox was approved by FDA and EMEA for the treatment of patients with transfusional iron overload -older than 2 years of age- as first line therapy, in 2005 and 2006 respectively. The primary objective of iron chelation is to maintain body iron at safe levels at all times but once iron is accumulated, the objective of iron chelation is to reduce tissue iron to the safe levels which is a slow process. The chelation regimen and dose (and frequency if the chelator is DFO) of the chelator(s) are mainly determined based on body iron burden, presence of myocardial iron and the transfusional iron loading rate. A proper monitoring of chelation has of importance for measuring response rate to a chelation regimen and providing dose adjustments to enhance chelation efficacy but avoid toxicity. Efficacy of a chelation regimen may show individual variability resulted from factors such as absorbtion and metabolism of chelator. Tolerability and compliance with the chelator are also individual variables effecting response to chelation. Understanding of advantages and limitations of chelators, accurately determining chelation needs of patients with iron overload and designing individualized chelation regimens with less toxicity but optimum efficacy should provide long-term survival and quality of life for patients with iron loading anemias. The goal of this review is to summarize current concepts in iron chelation therapies based on the considerable amount of prospective data obtained by clinical studies.

INITIAL TRIALS OF A NOVEL ORAL IRON CHELATOR

Ellis J. Neufeld

Egan Family Foundation Chair in Transitional Medicine, Hematology-Oncology, Children's Hospital Boston, Mass., USA

Chelators commercially available in 2011 effectively maintain iron balance in the majority of thalassemia patients. However deferoxamine, deferasirox and (where approved) deferiprone each have disadvantages (e.g. route of administration, tolerability, adherence, side effect profile) and worldwide, suboptimal chelation is common. Combined or alternating use of current chelators is insuf-

ficiently studied. The state of the field leaves ample room for novel chelators. FBS0701 (Ferrokin Biosciences) is a desferithiocin derivative currently in Phase 2 clinical development. The Phase 2 study is the fourth clinical study aimed at understanding the pharmacokinetics, safety, patient tolerability and activity of this agent for the treatment of transfusional iron overload. Pharmacokinetic parameters have been determined in both healthy volunteers and iron overloaded adults following single or multiple doses of FBS0701. With the exception of fraction excretion of oral dose in the urine, which was greater in healthy male volunteers than in iron, overloaded patients, there were no significant differences in pharmacokinetic parameters. Analysis of the plasma concentration and urinary excretion data in iron overloaded patients receiving FBS0701 after oral administration of 3 mg/kg, 7 mg/kg, 14 mg/kg, 29 mg/kg, and 36 mg/kg (as active drug) once-daily for seven (7) days indicates linear pharmacokinetics over that range of doses. There were no apparent dose dependencies with respect to Tmax, CL/F, Vz/F, or t½. The mean elimination half-life was ~15 hours and approximately 44% of the oral dose was recovered in the urine unchanged. CL/F appears to be directly related to body weight, suggesting that weight based dosing may be appropriate for FBS0701. Clearance was proportional to LIC over a three-fold range. Urinary iron excretion was dose proportional but deminimus. CTP-04 is a one-year Phase 2 randomized, open label study of FBS0701 initiated in September 2010. The primary objective is to evaluate safety, tolerability and efficacy based on clinical and MRI assessments of two doses of FBS0701 with a view to identifying a differential pharmacodynamic dose response to two dose levels (14 and 29 mg/kg/d of active drug). The study is fully enrolled with 50 patients. Primary outcome data are anticipated in the summer of 2011.

IRON CHELATION: COMPLIANCE AND CHALLENGES

Ahmet Varoglu

Northern Cyprus Thalassaemia Society

Thalassemia is an inherited blood disorder which in its most severe form it causes life threatening anaemia. It creates the need for life-long blood transfusions that result in iron overload in the body. Iron chelation agents are therefore necessary for the excretion of the excess iron from the body. Iron overload causes organ damage (liver, endocrine glands and bones); growth failure and cardiac failure. There are three main available chelating agents: 1) Desferrioxamine (DFO) is one of the chelating agents which has been available to use for more that 30 years. It has a short half life of approximately 20 minutes, so it must be given by continuous infusions and is administrated 8-12 hrs/day for 5-7 days/week. 2) Deferiprone (DFP) has been patented in 1982 and was licensed in EU in 1999. It is taken 3 times/day orally. 3) Exjade (ICL 670) it is licensed in the USA and the EU and it is given as once a day. All three chelating agents have different adverse side effects and patients have to comply with iron chelation treatment despite these side effects. Compliance is the state or act of conforming with or agreeing to do something and doctor's play an important role in helping and motivating patients to comply with their treatment. Thalassemia patients are asked to comply with all or many of the following treatments: blood transfusion; iron chelation; HCV/liver disease treatment; bone treatment; diabetes; cardiological monitoring; fertility treatment; endocrinological treatment; psychosocial issues and specific other complications. Although treatment has been getting better over the years, patients are still non compliant and there are a number of factors for that: non-acceptance of their condition; pain; forget to take their drugs; adverse side effects; feelings of being different; body image damage; get tired of following their treatment; lack of availability of drugs; cost of drugs; ignorance of parents.

THE ECONOMICS OF IRON CHELATION IN DEVELOPING COUNTRIES

Suthat Fucharoen

Thalassemia Research Center, Institute of Molecular Biosciences, Mahidol University, Nakornpathom, Thailand

Thalasseamia is one of the common genetic disorders. A genetic defect causes reduction of the globin chains leading to chronic haemolytic anaemia from birth. The mainstay of treatment is blood transfusion to maintain adequate levels of the haemoglobin. Secondary iron overload in β-thalassemia patients is secondary to multiple blood transfusions and increased iron absorption. Excesses iron potentially catalyses free-radicals generation and impairment in cellular function and integrity. Extensive iron-induced injury develops in the heart, liver, pancreas and endocrine system. In regularly transfused patients, in the absence of iron-chelation therapy, death from iron-induced heart failure occurs by the mid-teenage years. Conventional treatment with parenteral iron chelator desferrioxamine improves mortality but it is too expensive for middle and low-income countries. Oral iron chelator such as deferiprone (L1) and deferasirox appears to be promising, however, it is still too expensive or need special monitor. Serum ferritin has been used for many years as a guide for chelation therapy. However, recent studies demonstrated that using serum ferritin or liver iron measurements as a monitor of iron-chelation intensive therapy would have been discontinued long before the iron had cleared from the heart. There is evidence of the value of myocardial T2* measurements by MRI for the detection of early cardiac iron overload which cannot be predicted by liver iron or serum ferritin and for the monitoring of iron-chelation therapy. The major problem is the expensiveness of MRI measurement. In conclusion, the problem of iron chelation in low-income countries may be summarized as follows: i) drug is not available in every countries that need the medicine; ii) cost; iii) education of doctor, parents/patients and local government about the benefit of iron chelation, iv) monitoring of its toxicity and adverse drug reaction. In the TIF conference in Dubai, in 2006, a group of experts had agree to send the document to the D-G of the WHO with a strong request that all chelators (currently available and those

that will be available in the future) be designated essential for the treatment of transfusion dependent anaemias.

THE IDEAL CENTRE FOR HAEMOGLOBINOPATHIES

Antonio Piga, Roberta Rossi, Simona Roggero

Thalassemia & Haemoglobinopathies Centre, Department of Clinical and Biological Sciences, School of Medicine and Hospital S. Luigi Gonzaga, University of Torino, Italy

First of all an ideal Centre for Haemoglobinopathies must be a dedicated unit, as in large multi-purpose units other conditions (i.e. oncology) absorb most resources. Dedicated but not isolated, as the staff requires a career structure with promotion opportunities to allow low turnover rate and continuity of care. In addition to patient care, the Centre should include facilities for screening of carriers, genetic counselling, prenatal diagnosis and drive local (regional, national) prevention programs within the health system. A minimum number of patients (≈50) is critical to allocate resources and develop specific services. Basic patient care facilities should allow free access to high quality blood products, iron chelators, and tests for monitoring and supportive care. Ideally any medical prescription should be driven only by clinical indication and not biased by economic restriction. A treating physician specially trained in haemoglobinopathies oversees all aspects of treatment, referring to specialists when indicated. Most commonly involved are nurse specialist(s), cardiologist, endocrinologist, reproduction endocrinologist, andrologist or gynaecologist, dental team, dietician, hepatologist, transplant specialist, psychiatrist or clinical psychologist, social worker. The staff must include a charge nurse who supervises the nursing staff. Regular meetings for case review, work organisation review and procedures, together with haemoglobinopathies-oriented CME should be facilitated. More important than the medical background (Pediatrics, Hematology, Internal Medicine) is the interest of the group in development of optimal care and research in chronic diseases and transitional medicine. The thalassemia unit should operate on an outpatient basis, with facilities for evening and night transfusion to minimize interference with patient's social life. Important specific services include trans-cranial Doppler ultrasound, MRI for iron assessment and rapid access to care where the Centre physician is available for consultation or sharing the responsibility of management in any condition like emergency, hospital admission for any acute condition, heart failure, intensive chelation, and stem cell transplant. Quality control procedures should take into account patient' opinion. A well-balanced interaction with any specific patients and parents association is crucial on the way to optimal achievement.

EMOTIONAL ASSISTANCE IN THALASSEMIA: PILOT IMPLEMENTATION OF A STANDARD PROTOCOL

Maria Teresa Veit, Rita de Cassia Macieira, Marilia Zendron, Helena Pedrosa, Silvana Cantu, Ana Paula Teixeira

ABRASTA – Associação Brasileira de Talassemia, São Paulo, Brazil

This study aims to describe The creation process of standard procedures to make possible multicentre studies related to emotional aspects of thalassemic patients, their families and caregivers; and the pilot phase of the routines implementation. The objectives defined to perform this goal are: i) develop routines to assess and manage/treat emotional issues; ii) adjust the ABRASTA - Brazilian Association of Thalassaemia computer system to the input of collected data and its compilation; iii) conduct a pilot implementation of the routines; iv) discuss the whole process and propose next steps. Forty patients were assisted following the above mentioned routines of psychological evaluation, follow-up assistance and management of specific emotional issues. Conclusions are that the routines are adequate to enable multicenter research to compare findings and develop specific interventions to thalassemia patients, their families and caregivers; information gathered through them is an important means of subsidizing medical doctors and other members of the professional team, both in the therapeutic planning and in the communication process with patients and families; finally, considering the nature of the information, psychologists and psychiatrists are the most indicated professionals to perform the assessment and the interventions related to emotional issues, due to their professional background, training and specific skills that allow a free and candid communication with the patients and their families.

FACING PSYCHOSOCIAL ISSUES IN THALASSEMIA

Chris Sotirelis

Thalassemia β major is a life-long inherited condition that affects people of Mediterranean, Middle Eastern, South and Far East Asian origin. Depending upon the cultural background, the chronic nature of this condition and the fact that it is blood related, impacts the psychosocial aspects of people living with this condition and their carers. Even the first days with a new baby, can be extremely traumatic for a couple at risk. Often they are waiting for the result of the neonatal test and other tests which will determine whether their baby is affected and the severity. But even those who already know, it is also a very anxious time as they are coming to terms with what it means to have a thalassemic child. We shall examine the issues behind living as a thalassemic through the decades. They may also have cultural worries about stigma associated with the condition, although that stigma could mostly be in the perception of the parents rather than based in reality. This can cause the parents to isolate the child; some do not even communicate about thalasse-

mia with their child's teachers or carers. As a result of these and other factors, the parents may be so anxious and worried they find it hard to mix and communicate with their peers and even other family members. We shall also be looking at what happens even beyond the early years, and as societies become more enlightened, the issues other people consider "normal" have somehow got to be "normalised" by thalassemics through a variety of adaptive mechanisms. In the end, every person can be "as normal as they want to be", although they will need to fight within themselves and society at large for it, perhaps more than they would otherwise need to.

QUALITY OF LIFE IN PATIENTS WITH THALASSEMIA

George Constantinou

Quality of life of any person has the following requirements: *Good child life; Good adolescence life; Success in school and university; Fulfilment at work; Family creation; Achieving one's inspirations.* A patient with thalassemia has the same parameters to achieve the same quality of life, but the success of such a patient does not only depend on their family's support or their willingness and capabilities to work hard throughout their life, but is also dependent on whether the State, Hospital, Treating Doctor, Nurses, Family and Society are willing to look at them as a complete human being and not only just as a number or a burden. It is necessary for good quality treatment, to be provided in a manner that allows patients to concentrate on their life and not on how to overcome treatment obstacles. Treatment for thalassemia has reached a stage where all patients should live to a normal retirement age with a lower burden and so have a *Normal Life* and G*ood Quality of Life*. Why is this not happening?

OVERVIEW OF HEPATITIS ASSOCIATED LIVER DISEASE IN THALASSEMIA AND ITS CURRENT MANAGEMENT

George M. Dusheiko

Royal Free Hospital and University College London Medical School, UK

Excessive iron absorbtion occurs in patients with thalassemia. Multiple transfusions can lead to secondary iron overload, although regular chelation therapy can delay but not completely avert the development of hepatic fibrosis. The iron loading that is encountered is similar to that seen in genetic haemochromatosis, but is exacerbation by the requirement for blood transfusions that lead to parenteral iron overload. Iron loading is observed at an early age, and necessitates aggressive prophylactic use of oral and parenteral iron chelation therapy with close regulation. Iron loading in the liver leads to liver damage, hypersplenism, thrombocytopaenia secondary to hypersplenism and portal hypertension. Patients with thalassemia major require lifelong chelation therapy to prevent iron induced organ damage. There have been concerns regarding the effect of deferiprone induced progression of hepatic fibrosis but these have not been substantiated. Chronic hepatitis B infection and C infection remain an important cause of morbidity in thalassemics worldwide. Before screening of blood, transfusion was a major risk factor for the acquisition of hepatitis C and many patients with thalassemia major became chronically infected. Thus these patients are at risk of developing hepatic fibrosis both from iron overload and from chronic HCV infection, emphasizing the need for effective antiviral therapy in addition to regular iron chelation. Coexistent hepatitis B or C infection can increase the risk of progressive fibrosis of the liver leading to cirrhosis, complications of cirrhosis, liver failure and hepatocellular carcinoma. Studies have shown that iron overload and hepatitis C infection are independent risk factors for liver fibrosis progression and their concomitant presence results in a striking increase in risk. Non invasive means of estimating fibrosis may assist in the identification of advanced liver disease. Between 25-35% of those chronically infected with hepatitis B develop progressive liver disease. Several stages of hepatitis B exist. Typically HBeAg positive patients are younger and may have high levels of hepatitis B replication, but relatively mild liver damage in the immunotolerant phase of the infection. Patients with pre-core mutant disease are anti-HBe positive, but replicate a variant of hepatitis B not expressing HBeAg. Inactive carriers of hepatitis B are anti-HBe positive, but have low levels of HBV replication and normal serum amintransferases. The goals of therapy are to reduce and preferably maintain suppression of HBV DNA to prevent sequelae of HBV infection. The advent of potent nucleoside analogues with low barriers to resistance has improved the outlook for patients with hepatitis. Cirrhosis develops within 10 years in about 10-20% of patients with chronic hepatitis C. Older age at infection, concomitant alcohol abuse, concurrent HBV or HIV infection and other illness may be important aggravating cofactors. The natural history after the first 20 years has not been fully defined in prospective studies. Combination treatment with interferon alpha (pegylated interferon) and ribavirin has been the mainstay of treatment. Iron overload does not prevent the possibility of a sustained virological response. The advent of new direct acting antiviral and host acting antivirals will hopefully translate into improved outcomes from hepatitis C in thalassemics. The toxicities and drug -drug interactions of these new compounds will require special assessment in thalassemia.

COMPLICATIONS AND MANAGEMENT OF THALASSEMIA INTERMEDIA

Maria Domenica Cappellini

"Ca' Granda" Foundation IRCCS, University of Milan, Italy

β thalassemia is an inherited disorder of hemoglobin synthesis wherein mutations of the β globin gene lead to various degrees of defective β chain production, an imbalance in α/β globin chain synthesis, ineffective erythropoiesis, and a spectrum of anemia. Extremely diverse phenotypes exist within the β thalassemia syndromes. At one end of the spectrum is β thalassemia minor, a clinically silent, mildly hypochromic and microcytic anemia. At the

other end is β thalassemia major (TM) which refers to those patients whose clinical course is characterized by profound anemia, who present to medical attention in the first year of life, and who subsequently require regular blood transfusions and iron chelation therapy for survival. The term β thalassemia intermedia (TI) was suggested to describe patients who had clinical manifestations that are too severe to be termed minor yet too mild to be termed major, although there remains substantial overlap between the three conditions. If left untreated, three main factors are responsible for the clinical sequelae of TI: ineffective erythropoiesis, chronic hemolytic anemia, and iron overload. The degree of ineffective erythropoiesis is the primary determinant of the severity of anemia, while peripheral (intra- and extravascular) hemolysis of mature red blood cells (RBCs) remains secondary. Ineffective erythropoiesis is also associated with skeletal deformities and osteopenia attributed to erythroid marrow expansion as well as compensatory extramedullary hematopoiesis (EMH) leading to tumor formation anywhere throughout the body. Hemolysis has mainly been associated with splenomegaly; however, recent evidence suggests that hemolysis, along with other factors, is also the hallmark of a hypercoagulable state in TI. Hypercoagulability justifies the high rate of thromboembolic phenomena in patients with TI and may explain other complications such as pulmonary hypertension (PHT) with secondary right heart failure (HF). Ineffective erythopoiesis and chronic anemia also lead to an increase in gastrointestinal iron absorption, resulting in non-transfusional iron overload (similar to patients with hemosiderosis), in the liver and less so in the heart. Involvement of the liver can eventually lead to cirrhosis and hepatocellular carcinoma. A recent report on 120 treatment-naive patients with TI revealed a significant role for advancing age (even among pediatric and adult patients) in acquiring clinical complications. The study demonstrated a decreasing trend in Hb level and a progressive increase in iron accumulation with advancing age. Thus, despite being considered as having a milder form of the disease at initial presentation and diagnosis, TI patients are still at risk of acquiring several serious complications with the passage of time, which warrants optimal and early intervention extremely essential.

TRANSFUSION REGIMENS IN THALASSEMIA INTERMEDIA

Zeynep Karakas

Istanbul University, School of Medicine, Department uf Pediatrics, Division of HematologyOncology, Thalassemia Unit, Istanbul, Turkey

Thalassemia intermedia (TI) is a heterogeneous disease, in terms of both clinical manifestations and underlying molecular defects. Some TI patients are asymptomatic until adult life, whereas others are symptomatic from early childhood. In contrast to patients with TM, patients with TI have less severe anemia and do not require transfusions during at least the first few years of life. Many patients with TI, especially older ones, have been exposed to the multiple long-term effects of chronic anemia and tissue hypoxia and their compensatory reactions, including enhanced erythropoiesis and increased iron absorption. Bone marrow expansion and extramedullary hemopoiesis lead to bone deformities and liver and spleen enlargement. In patients with TI, the heart is primarily affected by, which is the leading cause of congestive heart failure (CHF). High CO resulting from chronic tissue hypoxia is the main contributing factor for pulmonary hypertension (PHT) and CHF. Therapeutic strategies in TI, are not clear and different criterias are used to decide the initiation of transfusion and chelation therapy, modulation of fetal hemoglobin production, and hematopoietic stem cell transplantation with individual basis. It seems that the majority of TI patients will be benefited if this kind of treatment is applied targeting prevention and not palliation of the anemia-induced complications. The clinical picture of well-treated TM patients with regular transfusion-chelation therapy is better from TI patients who have not received transfusions or have occasionally received transfusions. The new oral iron chelators and the magnetic resonance imaging application for early detection of heart iron load are promising for further improvement on survival. There are a significant role of early blood transfusion to prevent and treat complications of commonly associated with TI as extramedullary erythropoiesis and thalassemic stigmata, autoimmune hemolytic anemia, leg ulcers, gallstones, *Pseudoxantoma Elasticum,* hyperuricuria and gout, and pulmonary hypertension. which are rarely seen in thalassemia major. Nowadays, indications of transfusion patients with TI are chronic anemia (Hb <7 g%), bone deformities, growth failure, extramedullary erythropoiesis, heart failure and pregnancy and prior to surgical procedures. Regular transfusion therapy is an important treatment modality for increasing quality of life in some patients with thalassemia intermedia during chıldhood.

COAGULATION AND THROMBOTIC RISK IN THALASSEMIA INTERMEDIA

Ali Taher

Department of Internal Medicine, American University of Beirut Medical Center, Beirut, Lebanon

Extremely diverse phenotypes exist within the thalassemia syndromes. The term thalassemia intermedia (TI) was first suggested to describe patients who had clinical manifestations that were too severe to be termed minor yet too mild to be termed major, although there remains substantial overlap between the three conditions. Our understanding of the molecular and pathophysiological mechanisms underlying the disease process in patients with TI has substantially increased over the past decade. Three main factors highlight the pathophysiology of TI, ineffective erythropoiesis, chronic anaemia/haemlysis, and iron overload secondary to increased intestinal absorption. However, the extreme diversity in phenotypic expression in TI patients led to a wide variation in observed clinical complications and management practices, which remain solely based on physician preferences rather than evidence-based guidelines. Among the clini-

cal complications of TI that were found to occur at a higher rate than in patients with TM are thromboembolic events (TEE). The largest epidemiological study to date analysed data from 8860 thalassemia patients (6670 thalassemia major [TM] and 2190 TI) and demonstrated that TEE occurred 4.38 times more frequently in TI than TM patients. The hypercoagulability in TI has been attributed to several factors including a procoagulant activity of haemolysed circulating red blood cells (RBCs), increased platelet activation, coagulation factor defects, depletion of antithrombotic factors, endothelial inflammation, among others. These factors have been observed at a higher rate in splenectomised patients. Clinical studies also confirmed that splenectomised TI patients have a higher incidence of TEE than non-splenectomised controls, and a higher rate of silent cerebral infarction in older patients. In the OPTIMAL CARE study, 73/325 (22.5%) splenectomised patients developed TEE compared to 9/259 (3.5%) non-splenectomised patient (P<0.001). A subanalysis of the OPTIMAL CARE study also demonstrated that splenectomised TI patients who will develop TEE might be identified early on by high-nucleated RBC and platelet counts, evidence of pulmonary hypertension, and transfusion naivety. Currently, there is lack of prospective clinical trials that evaluate the role of antiplatelets, anticoagulants, or transfusion therapy in the prevention of TEE in thalassemia patients; such trials are called for.

CARDIAC FAILURE IN β-THALASSEMIA: DIAGNOSIS, PREVENTION AND MANAGEMENT

Athanasios Aessopos

First Dept. of Internal Medicine, Athens University Medical School, Laiko Hospital, Athens, Greece

Heart failure always represented and still remains the leading cause of mortality in beta (β)-thalassemia, despite the therapeutic advances and the considerable amelioration of prognosis accomplished over the last decades. High cardiac output due to chronic anemia and myocardial iron overload due to repetitive blood transfusions are the two main pathogenetic mechanisms causing heart failure in β-thalassemia. In regularly treated thalassemia major patients, left ventricular dysfunction, resulting mainly from myocardial siderosis, is considered to be the primary cause of heart failure and thus the prevention, early recognition and effective management of iron overload is of key importance. However, the spectrum of cardiovascular complications that may ultimately lead to heart is wide and should be individually investigated in each one of the patients. Echocardiography is the main modality used for the regular follow-up and screening of asymptomatic patients and for the evaluation of patients with cardiac symptoms, while the T2* relaxation time provided by magnetic resonance imaging allows the accurate identification and quantification of myocardial iron burden and thus the proper guidance of iron chelation therapy.

References

1. Aessopos A, Farmakis D, Deftereos S, et al. Thalassemia heart disease: a comparative evaluation of thalassemia major and thalassemia intermedia. Chest 2005;127:1523-30.
2. Aessopos A, Farmakis D, Hatziliami A, et al. Cardiac status in well-treated patients with thalassemia major. Eur J Haematol 2004;73:359-66.
3. Aessopos A, Farmakis D, Karagiorga M, et al. Cardiac involvement in thalassemia intermedia: A multicenter study. Blood 2001;97:3411-3416.
4. Aessopos A, Farmakis D, Andreopoulos A, Tsironi M. Assessment and treatment of cardiac iron overload in thalassemia. Hemoglobin 2009;33 Suppl 1:S87-92.
5. Aessopos A, Kati M, Farmakis D. Heart disease in thalassemia intermedia: a review of the underlying pathophysiology. Haematologica 2007;92:658-65.
6. Kremastinos DT, Farmakis D, Aessopos A, et al. Beta-thalassemia cardiomyopathy: history, present considerations, and future perspectives. Circ Heart Fail 2010;3:451-8.
7. Anderson LJ, Holden S, Davis B, et al. Cardiovascular T2-star (T2*) magnetic resonance for the early diagnosis of myocardial iron overload. Eur Heart J 2001;22:2171-9.
8. Pennell DJ, Berdoukas V, Karagiorga M, et al. Randomized controlled trial of deferiprone or deferoxamine in beta-thalassemia major patients with asymptomatic myocardial siderosis. Blood 2006;107:3738-44.
9. Tanner MA, Galanello R, Dessi C, et al. Combined chelation therapy in thalassemia major for the treatment of severe myocardial siderosis with left ventricular dysfunction. J Cardiovasc Magn Reson 2008;10:12.
10. Kirk P, Roughton M, Porter JB, et al. Cardiac T2* magnetic resonance for prediction of cardiac complications in thalassemia major. Circulation 2009;120:1961-8.

DIAGNOSIS OF IRON OVERLOAD AND HEART DISEASE BY MAGNETIC RESONANCE IMAGING

John C. Wood

Division of Cardiology, Children's Hospital of Los Angeles, CA, USA

The use of magnetic resonance imaging (MRI) to estimate tissue iron was initiated nearly three decades ago but has only become a practical reality in the last ten years. MRI is most often used to estimate hepatic and cardiac iron in patients with thalassemia or sickle cell disease and has largely replaced liver biopsy for liver iron quantification. The ability of MRI to image extrahepatic organs has really transformed our understanding of iron mediated toxicity in transfusional siderosis. For decades, iron cardiomyopathy was the leading cause of death in thalassemia major, but it is now relatively rare in centers with regular MRI screening. Early recognition of cardiac iron loading allows more gentle modifications of iron chelation therapy prior to life threatening organ dysfunction. Serial MRI evaluations have demonstrated differential kinetics of uptake and clearance among the difference organs of the body. Although elevated serum ferritin and liver iron concentration increase the risk of cardiac and endocrine toxicities, extrahepatic iron deposition and toxicity occurs in many patients despite having low total body iron stores; there is no safe liver iron level in chronically transfused patients. Instead, the type, dose, and pattern of iron chelation therapy all contribute to whether cardiac iron accumulation will occur. These observations, coupled with the advent of increasing options for iron chelation therapy, are allowing clinicians to more appropriately tailor chelation therapy to individual patient

needs, producing greater efficacy with fewer toxicities. With the decline in cardiac mortality, future frontiers in MRI monitoring including better prevention of endocrine toxicities, particularly hypogonadotropic hypogonadism and diabetes. These organs also serve as early warning signals for inadequate control of non-transferrin bound iron, a risk factor for cardiac iron loading. Thus MRI assessment of extrahepatic iron stores is a critical monitoring tool for chronically transfused patients. Further prospective work is necessary to determine whether markers of endocrine and exocrine pancreatic function can be used as surrogates of cardiac risk in regions where MRI is not available.

PULMONARY HYPERTENSION: AN EMERGING RISK IN HEMOGLOBIN DISORDERS

Dimitrios Farmakis

First Dept. of Internal Medicine, Athens University Medical School, Laiko Hospital, Athens, Greece

Pulmonary hypertension (PH) is one of the main cardiovascular complications in hemoglobinopathies and is considerably implicated in patients' morbidity and mortality. In thalassemia intermedia, PH is found in about 60% of traditionally managed patients and represents the main cause of heart failure. In sickle cell anemia, PH is encountered in one third of patients and has been found to be a strong and independent predictor of mortality, while in sickle thalassemia, PH is generally less frequent and severe. The pathophysiology of PH in hemoglobinopathies is multifactorial and several mechanisms seem to be implicated, including a complex vasculopathy, hypercoagulability and elastic tissue defects, all associated with chronic hemolysis, high output state due to chronic anemia, as well as left heart dysfunction, pulmonary disorders and thromboembolic complications. Echocardiography is the most useful tool for patients' screening on a regular basis, while the diagnosis of PH should always be confirmed by right cardiac catheterization. The proper management of the disease itself with hematological modalities such as blood transfusions combined with iron chelation or hydroxyurea, is the most effective approach for the prevention and treatment of hemoglobinopathy-associated PH. Antithrombotic agents should also be considered while the value of novel agents used in the treatment of pulmonary arterial hypertension, including endothelin antagonists or phosphodiesterase-5 inhibitors, is not yet established in patients with hemoglobinopathies.

References

1. Farmakis D, Aessopos A. Pulmonary hypertension associated with hemoglobinopathies: Prevalent but overlooked. Circulation 2011;123:1227-32.
2. Aessopos A, Farmakis D. Pulmonary hypertension in -thalassemia. Ann N Y Acad Sci 2005;1054:342-9.
3. Aessopos A, Farmakis D, Karagiorga M, et al. Cardiac involvement in thalassemia intermedia: A multicenter study. Blood 2001;97:3411-3416.
4. Gladwin MT, Sachdev V, Jison ML, et al. Pulmonary hypertension as a risk factor for death in patients with sickle cell disease. N Engl J Med 2004;350:886-95.
5. Aessopos A, Farmakis D, Trompoukis C, et al. Cardiac involvement in sickle beta-thalassemia. Ann Hematol 2009; 88:557-64.
6. Aessopos A, Kati M, Farmakis D. Heart disease in thalassemia intermedia: a review of the underlying pathophysiology. Haematologica 200792:658-65.
7. Aessopos A, Farmakis D, Tsironi M, et al. Endothelial function and arterial stiffness in sickle-thalassemia patients. Atherosclerosis 2007;191:427-32
8. Aessopos A, Farmakis D, Loukopoulos D. Elastic tissue abnormalities resembling pseudoxanthoma elasticum in beta thalassemia and the sickling syndromes. Blood 2002;99:30-5
9. Taher AT, Musallam KM, Karimi M, et al. Overview on practices in thalassemia intermedia management aiming for lowering complication rates across a region of endemicity: the OPTIMAL CARE study. Blood 2010;115:1886-92.

CARDIAC ARRHYTHMIA: DIAGNOSIS, MANAGEMENT AND NEW TECHNOLOGIES

Malcolm Walker

Consultant Cardiologist the Heart Hospital and University College Hospital, London, UK
Clinical Director, Hatter Cardiovascular Institute, University College Hospital, London, UK

The normal co-ordinated contraction of the heart with its minute to minute responsiveness to demand depends on the electrical excitability of cardiac cells and their careful organisation into regions of spontaneous electrical discharge (normally the sinus node), separated by areas of insulation, or non-conduction, as well as distinct anatomical areas of slow electrical impulse propagation (the atrio-ventricular node) and finally systems of rapid impulse propagation, the His Purkinje network. Abnormalities of cardiac rhythm, detected by changes in the rate or regularity of the pulse were noted in antiquity and their association with disease was known to Hippocrates. The early twentieth century saw Einthoven's string galvanometer developed into the now ubiquitous electrocardiograph (ECG or EKG) and the clinical definition of the abnormalities of cardiac conduction and rhythm. Broadly arrhythmia fall into two major categories: the bradycardias, where the heart beats too slowly (pragmatically defined as <60 bpm) and tachycardias, where the heart beats too fast, for the circumstance (>100 bpm, at rest). Arrhythmias are common and may be encountered even in structurally and functionally normal hearts. They may also be the first symptom of serious heart disease, but the severity of symptoms does not always differentiate between minor clinical problems and potentially life threatening complications. The clinician's role is to navigate between these two radically different scenarios, by detecting and characterising the arrhythmia, usually by some form of ECG monitoring, including at times intracardiac electrophysiological testing (EP) and, most importantly, determining the underlying "health" of the heart. This factor is particularly important in the haemoglobinopathy population, where knowledge of the cardiac status, in terms of function and structural integrity plus an assessment of myocardial iron content by magnetic resonance scanning (cMR), is mandatory. This knowledge allows rational, targeted therapy, particularly where the arrhythmia may be eradicated by intensification of chelation therapy, often without the need to consider specific cardiac anti-arrhythmic drugs. Atrial fibrillation (AF) was previously associated with severe myocar-

dial iron overload, often signalling the onset of cardiac failure. In the current situation, in our clinic, AF is now being seen increasingly commonly in older thalassemia patients without evidence of important myocardial iron overload. It may prove to be a difficult clinical management problem requiring the use of EP techniques in severe cases. These patients are at potential risk of stroke and must be offered anti-coagulation, until the AF is permanently eradicated. Severe bradycardias and heart block, although encountered are very rare in our experience, but may require pacemaker therapy, with significant implications for the use of cMR scanning.

OVERVIEW OF CURRENT AND EMERGING ISSUES IN ENDOCRINOLOGICAL COMPLICATIONS OF THALASSEMIA

Vincenzo de Sanctis

Pediatric and Adolescent Outpatient Clinic - Quisisana Hospital, Ferrara, Italy

Clinical advances in the treatment of thalassemia major (TM) patients have helped to increase substantially the life expectancy of patients. The TM patients today represent the first generation of adult thalassemics. As patients enter puberty, they begin to experience a variety of endocrine abnormalities, presumably the results of chronic anaemia and tissue iron deposition from the chronic transfusion therapy. In patients with TM, the anterior pituitary gland is particularly sensitive to free radical stresses. It has been reported that the GH deficiency (GHD) may be secondary to either pituitary or hypothalamic dysfunction. The duration of the disease, the patient's age and the severity of iron overload are the most important factors responsible for the defect of growth hormone (GH) secretion. Recent reports have documented a frequency of severe growth hormone deficiency in 13-32% of adult patients with TM. All of these patients underwent GH-releasing hormone (GH-RH) plus arginine (ARG) testing.We found a high prevalence of severe GHD in adult TM patients after glucagons stimulation test (GST). This percentage is higher compared to previous reports and may be related to the older age (37.8±8.5 years) of our patients and to the GH stimulation test employed in our study.The prevalence of impaired adrenal function in TM patients (from 0-33%) depends on the age of the population studied, duration of blood transfusion, iron overload and procedures used for the evaluation of the diagnosis, has been reported in the literature, although clinical adrenal insufficiency (AI) is rare. In our study, all TM patients had morning normal cortisol concentrations. But 17 out of 25 patients (68%) had an impaired peak cortisol response after GST and one TM patient had peak cortisol response compatible with AI. Thyroid dysfunction has been observed in 13-60% of patients, but its severity is variable in different series. Acquired central hypothyroidism (CH) is a rare complication. Acquired CH has been observed in 4 out of 1590 (0.25%) TM patients, from May 1972 to October 2010, followed in Ferrara. Our recommendation is that if the level of FT4 is in the lower one-third of the normal range, then the patient should be started on levothyroxine (L-T4), but only after having excluded a concomitant adrenal insufficiency. If the level of free thyroxine is not consistently low, then we follow the patient frequently. In the management of CH patients the best way to monitor hypothalamic-pituitary-thyroid function is the measurement of serum FT4.

ENDOCRINE CONCERNS AND QUALITY OF LIFE IN THALASSEMICS

Unver Ahmet

TADAD Thalassaemia Patient Parent Association Headquarters General Secretary, Istanbul, Turkey

I was born in 13 April 1979 in Istanbul, Turkey. My grand grandparents origins from Crete. When I was 5-year-old, my skin color was a little bit pale and I had eating disorder. My parents got suspicious and take me to a doctor. After the blood tests, I was diagnosed thalassemia major and started to transfuse blood every month. And also started to use Desferral 5 days in a week. But the main problem was neither my parents nor me knew anything about thalassemia. In 1989, my parents participated a meeting of TADAD (Thalassaemia Patient Parent Association) and became members of this organization. In 1991 I went to Whittington Hospital in London to see Dr. Wonke, who decided that I am not a major thalassemia patient, but my disease is an intermediate form. I had splenectomy operation. After that operation my blood transfusion period changed from one month to three months and my Desferral usage reduced to 3 nights a week. When I was 20-year-old, Dr. Wonke suggested me to start hydrea and by the help of hydrea, I transfused blood every six months. I am still using hydrea 3 capsules a day. In 2003 I met with my wife. After 3 years we decided to have a kid but no matter what we have tried, we could not. The gynecologist Dr. Semra Kahraman diagnosed that I also have azospermia. She suggested the *in vitro* fertilization technique to have kids. She also told me that is an endocrine complication. After a small operation they found sperms and fertilized them with my wife's eggs in a laboratory. Nine months later my son had born, who is now 1.5-years old. Due to my business I need to travel a lot. During these travels I got tired very quickly. Actually endocrine problems started way back from my childhood. Imagine you are a little kid and want to play with your friends, you want to run with them play ball with them but you couldn't. You have to stay at home or sit on the side and watch it is a hard thing to do for a kid. When you became a teenager you couldn't drink with your friends do any kind of sports. This has also psychological problems. After a while you fed up with this illness, tests, blood transfuses medication usage and tries to run away from all this, and became an antisocial person. I suggest that besides all of these problems life is still good you should hang on to it.

ENDOCRINE INVESTIGATION AND FOLLOW UP IN THALASSEMIA: TIME FOR SPECIFIC GUIDELINES

Nicos Skordis

Pediatric Endocrine Unit, Makarios Hospital, Nicosia, Cyprus

Iron deposition in endocrine glands in patients with thalassemia major (TM) is the main cause of endocrine complications. The child with TM has a particular growth pattern, which is relatively normal until age 9-10 years; after this age a slowing down of growth velocity is observed. The pathogenesis of growth failure is multifactorial as seen in Figure 1.

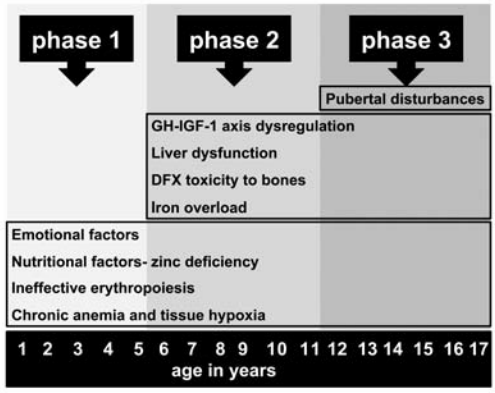


Figure 1. Three phases of growth according to age of presentation, which have different causes – the multifactorial origin of growth failure in thalassemia.

The abnormal upper-to-lower segment ratio can be attributed to delayed puberty and desferrioxamine (DFX) toxicity. *Treatment:* anemia, folate deficiency and hypersplenism are traditional causes of poor growth in TM where transfusion is not regular. In countries where DFX is regularly used, this is a major cause of growth retardation and should be monitored. In peri-pubertal patients, hypogonadism should be carefully investigated before starting treatment with growth hormone (GH). GH treatment often with high dose is not always as effective as expected and may result in decreased insulin sensitivity and abnormal glucose tolerance. Therefore GH should be viewed as not a panacea and judiciously given in selected cases with proven GH deficiency. Delayed puberty and hypogonadism are the most obvious clinical consequences of iron overload. Delayed puberty is defined as the complete lack of pubertal development in girls by the age of 13 years, and in boys by the age of 14 years. Hypo - gonadism is defined in boys by the absence of testicular enlargement (less than 4 mL), and in girls by the absence of breast development by the age of 16 years. Most women with TM present with primary amenorrhea, whereas secondary amenorrhea will invariably develop with time especially in patients poorly compliant to chelation therapy. Ovarian function of these women is normal as they produce the expected number of ova after stimulation therapy. Damage of the ovaries by iron deposition is rarer and is more likely to appear in women of 25-30 years of age because of high vascular activity on the ovaries at this age. The treatment depends on factors such as age, severity of iron overload, damage to the hypothalamo-pituitary-gonadal axis and chronic liver disease. Sex steroids are the optimal therapeutic regimen – in females' estrogens with progesterone to maintain the normal menstrual cycle and in males testosterone preparations. Induction of spermatogenesis has been successfully achieved with the combination of hormonal preparations mimicking FSH and LH. The treatment of hypogonadism is a complex issue as sex steroids have a great impact on the quality of life of the adult thalassemic and each patient has to be assessed individually. Therefore guidelines are needed in regard to the best therapeutic regimen and the duration of treatment. The results of our recent study (De Sanctis *et al.*) are consistent with a high prevalence of severe GH deficiency in adult TM patients (54.5%). These findings raise a vital question regarding GH administration, as GH in the adults is involved in numerous biologic functions, most importantly that of cardiac morphology and function.

PAIN IN THALASSEMIA: AN EMERGING COMPLICATION

Patricia J. Giardina

Paediatric Haematology, Weil Cornell Medical College, New York, NY, USA

There is now a growing awareness that many thalassemia subjects both transfused (Major/TM) and non-transfused (intermedia/TI) suffer from longstanding bone disease, reduced or low bone mass (osteopenia or osteoporosis), fractures and bone pain. The prevalence of low bone mass, fractures and pain has been recently documented among all thalassemia syndromes by the North American Thalassemia Clinical Research Network (TCRN) and studied by other international groups. Historically bone disease in thalassemia has been associated with the marked osseous changes and in particular the facial and limb deformities, pathological fractures, premature epiphyseal closure. The pathogenesis of bone disease has been attributed to the underlying marrow expansion of medullary bone caused by the massive ineffective erythropoiesis and subsequent cortical thinning as well as metabolic deficiencies and endocrine dysfunction secondary to iron overload and in some instances iron chelation. The advent of hypertransfusion therapy to maintain near normal pretransfusion hemoglobin levels resulted in the reduction or prevention of bone deformities. Therefore the detection of low bone mass, the high prevalence of fractures and pain in regularly transfused and well-chelated subjects over the last few decades was quite unexpected. I will review the outcomes of the TCRN studies of bone disease to reveal the nature of bone disease across all ages and thalassemia syndromes and identify the prevalence and nature of fractures and pain and briefly discuss the pathogenesis and therapeutic options of bone disease in thalassemia.

BISPHOSPHONATES – WHEN TO START AND WHEN TO STOP TREATMENT

Ali Kemal Topaloglu

Cukurova University, Department of Pediatric Endocrinology, Adana, Turkey

Osteopenia or osteoporosis is seen in approximately half of the even well-treated thalassemia major patients. Several factors are implicated in thalassemia-associated bone disorder including accompanying diabetes mellitus, hypothyroidism, hypoparathyroidism, accelerated hemopoiesis with progressive marrow expansion, iron toxicity on osteoblasts, and several polymorphisms in bone-related genes. Pathogenesis involves reduced osteoblastic activity accompanied by an even greater increase in bone resorption. Bisphosphonates are potent inhibitors of osteoclastic bone resorption. They mainly act by inhibiting osteoclastic development and activity. Bisphosphonates are considered among the in mainstays the management of osteoporosis in these patients along with improved nutrition, hormone, vitamin and nutrient replacements and specific thalassemia treatments. However, details of how to use bisphosphonates including when to start/stop have not been established. It is plausible to individualize the bisphosphonates treatment as the degree of osteopenia/osteoporosis and the age it clinically emerges vary among patients. Also, caution should be exercised for potential long-range side effects as these drugs have been shown to be active several years onwards.

PAIN AND BONE DISEASE: A PATIENT'S VIEW

Loris Brunetta

The improvement in life expectancy of thalassemia patients due to diagnostic and therapeutic approaches adopted in recent decades has shifted the focus on monitoring and on management of complications. Main complications, related to transfusion therapy, the iron overload on the liver and on heart, have been investigated for years. This led to a significant improvement in both quality of life and life expectation for patients. Adult thalassemia patients reported pain with increasing frequency and in some cases they complained the occurrence of fractures due to osteoporosis. A study on quality of life conducted in Genoa, Italy, in 2001 revealed several patients suffering from a widespread pain. According to this study, about 40% of patients with thalassemia major and 15% with thalassemia intermedia reported a constant back pain and a widespread state of malaise due to the presence of pain. Data from more recent studies increases this percentage up to 69%. Until a few years ago, patients had no adequate therapeutic responses perhaps due to the fact that the presence of osteoporosis was not usually associated with the presence of pain. Furthermore therapies administered were poorly effective to control adequately the pain. The constant presence of the pain forced patients to spend several days away from their job, causing social and psychological problems and a decreasing self-esteem. From the clinical point of view the mechanism that leads to osteoporosis is still unclear, however today something more than in the past has being done. The use of bisphosphonates, the only currently available drugs, is only partially effective probably due to subjective patient response to treatment. According to other studies, though, the use of these drugs is improving the situation. Preliminary results of another study held in Genoa seem to indicate a marked improvement in the perception of pain due to osteoporosis and a general improvement in the patients' perception of their state of health. As reported in every development of therapies in the field of thalassemia, it is important to follow a multidisciplinary approach. This means to involve different specialists, able to design and run clinical studies to identify the mechanisms of the disease. The main objective should be to find the optimal management of the problem in order to prevent the osteoporosis, whether it is possible, and to reduce the presence of disabling pain in patients.

OVERVIEW OF BONE DISEASE RESEARCH IN THALASSEMIA – CURRENT AND FUTURE

Evangelos Terpos

Department of Clinical Therapeutics, University of Athens School of Medicine, Athens, Greece

Osteoporosis represents a prominent cause of morbidity in patients with thalassemia major (TM). The delay in sexual maturation, the presence of diabetes and hypothyroidism, the parathyroid gland dysfunction, the progressive marrow expansion, the iron toxicity on osteoblasts, the iron chelators, the deficiency of growth hormone (GH) or insulin growth factors (IGFs) have been identified as major causes of bone loss in TM. Dynamic bone formation histomorphometry studies established reduced bone formation rate in TM patients, which is thought to-date to be mainly the result of iron poisoning in osteoblasts and/or the result of reduced function of GH and IGF-1 axis. However, novel molecules seem to be implicated in osteoblast dysfunction in TM. Dickkopf-1 (Dkk-1) is a Wnt signaling inhibitor that inhibits the osteoblast differentiation and function. Sclerostin is another Wnt inhibitor which is produced by osteocytes and inhibits osteoblast-driven bone formation. Both Dkk-1 and sclerostin were found to be elevated in TM patients. Furthermore, recent data support that the reduced osteoblastic activity is accompanied by a comparable or even greater increase in osteoclast function. This increased osteoclast activity is due to an imbalance in the receptor-activator of nuclear factor-kB ligand (RANKL)/osteoprotegerin (OPG) system as the ratio of sRANKL/OPG is elevated in the serum of TM patients with osteopenia/osteoporosis. Moreover, the overproduction of cytokines that are involved in osteoclastogenesis, such as interleukin (IL)-1, IL-6 and transforming growth factor-β and tumor necrosis factor-α is present in TM. These data suggest that in addition to our current management of osteoporosis in TM, novel drugs that target RANKL, such as denosumab, which has been licensed by FDA and EMEA for the treatment of postmenopausal osteoporosis, and antibodies against Dkk-1 or against sclerostin may be future agents for the effective control of bone loss in thalassemia.

SICKLE CELL DISEASE: AN OVERVIEW OF PATHOGENESIS AND TREATMENT

Abdullah Kutlar

Medical College of Georgia, Augusta, GA, USA

The last three decades have witnessed a tremendous progress in our understanding of mechanisms contributing to the pathogenesis of sickle cell disease (SCD). This has led to advances in therapeutic approaches, especially in developed countries, with significant improvement in life expectancy to around the mid-50s. Despite this progress, huge global challenges remain: cure remains elusive; significant challenges to public health, especially in the developing world; health disparity, even in developed Western countries. Progress in deciphering the mechanisms involved in the pathology of SCD has changed our understanding. The disease is no longer thought of as a process characterized by episodic microvascular occlusion leading to acute complications between which the affected individuals have a so called "steady state," a state of relative normalcy. Rather, we now think of SCD as a chronic illness with an ongoing inflammatory state and chronic hemolysis punctuated by episodes of acute complications with heightened inflammation and microvascular occlusion ultimately leading to chronic organ damage, significant morbidity, and early mortality. Such an understanding requires a paradigm shift in our approach to therapy with emphasis changing from interventions aimed at acute symptomatic relief to disease modifying modalities. A major area of intense research has focused on unraveling the basis of phenotypic/clinical heterogeneity of SCD. Beyond the well known genetic modifiers (high Hb F, co-existing α-thalassemia), during the past decade of the human genome era, a large number of studies have tried to decipher the non-globin genetic modifiers of SCD. While some interesting results have emerged from candidate gene approaches and more recently from genome wide association studies, this field is still in its infancy. In terms of therapy, with the exception of stem cell transplantation (limited availability) and gene therapy trials (very early stages), there are no available curative strategies. Thus, disease modifying approaches will form the mainstay of treatment for the foreseeable future. Foremost among these are anti-switching (Hb F inducing) therapies. Hydroxyurea has proven to be an effective treatment modality for the majority of patients with SCD. Recent trials of IMIDs, histone deacetylase inhibitors, and DNA methyl transferase 1 inhibitors appear promising. Approaches targeting downstream effects of "sickling" (RBC cation loss and dehydration, cell adhesion, inflammation, hemolysis) may be important; however, lessons from clinical trials in the past decade suggest that efficacy as single agents may be very limited and may best be used in conjunction with anti-switching therapies.

PREVENTION AND MANAGEMENT OF STROKES IN SICKLE CELL DISEASE

Yurdanur Kilinç

Çukurova University, Faculty of Medicine, Department of Pediatric Hematology, Balcalı, Adana, Turkey

Sickle cell disease (SCD) is one of the most common hemoglobinopathies in the world which causes stroke by the physiopathological events beginning with vasculopathy in circulating blood. The stroke is four times prevalent in homozygous SS(SCD) patients than the other SCD syndromes (US Department of Health and Human services) and it is about 0.5-1.0% in children. Especially in childhood, anatomic and phsiological abnormalities of CNS in childhood may be a predisposing factors even if they seem to be normal neurologically. Although, mostly, it affects to the distal segments of the internal carotid artery, also middle and anterior segments of the cerebral arteries are involved. The presence of cerebral infarction with transcranial Doppler ultrasound is seen as a high risk factor for stroke. Stroke could be prevented by periodical ultrasound examination and selective red blood cell transfusions are the first steps for the prevention of stroke. The stroke is the most troublesome complication of sickle cell anemia.The management of stroke depends on the manifestations and age of the patient. When the oxygene supply to the brain is insufficient, brain dysfunction occurs. Brain ischemia findings are hemiparesis, visual and language disturbances, seizures, altered sensation, mentation and alertness. Because of high oxygene demand in children, the child with SCD who also has anemia is of particular risk. The most frequent clinical findings are hemiparesis, speech defects, focal seizures, gaiting disturbancies. If there is also intracranial bleeding, sudden severe headache, sometimes associated with neck pain, vertigo, syncope, nistagmus, ptozis, meningismus and photofobia. The indication for surgical intervention is based upon Magnetic Resonance Angiography - MRA). The diagnostic approach is done with CBC, reticulocyte count, ICSs %, trans cranial doppler, computarized tomography, magnetic resonance imaging, MRA, and cerebral arteriography. In treatment, fluid supplementation, chronic transfusion programme at least for 6 months with exchange transfusion or erythrocytapheresis for reducing the HbS under 30%. Hydroxyurea should be given to increase HbF. In selected cases, stem cell transplantation will be lifesaving.

MANAGEMENT OF THE ACUTE PAINFUL CRISIS IN SICKLE CELL DISEASE

Paul Telfer

Consultant Haematologist, The London and St. Bartholomew's Hospital, London, UK

The acute painful crisis is the commonest acute complication in children and adults with homozygous sickle cell disease. The majority of episodes in older children and adults are managed at home, but more severe episodes, and those presenting with co-morbidities require treatment in hospital. Principles of acute hospital care include: i) Immediate effective analgesia (preferably within 5 min

from arrival). ii) Sustained analgesia to continue until pain beings to settle. iii) Withdrawal of analgesics as the pain settles. iv) Regular monitoring by nursing and medical staff to identify adverse effects of analgesics and development of additional sickle cell complications. There is no single analgesic drug which is acceptable and suitable in all settings. We have found intranasal diamorphine very effective for immediate analgesia, and the large majority of children can then be managed with an oral regime of morphine. Short acting morphine (oromorph) needs to be given pro-actively and more frequently during the first 6-12 hours of the crisis. We add in long-acting morphine (MST) at 6 hours if the pain is not well controlled, and this is a very effective analgesic for short-term (1-7 days) background pain control. Intravenous opiates (via patient/nurse controlled analgesic devices) are used in many units. Analgesia during the acute chest syndrome is a situation where a short acting intravenous opiate is required. There is no risk of opiate dependency if acute crises are managed according to these principles.

FERTILITY AND PREGNANCY ISSUES IN THALASSEMIA

Ratna Chatterjee, Rekha Bajoria

Institute For Women's Health, University College Hospital, London, UK

Advances in the primary care of β-thalassemia major by optimal blood transfusions and chelation therapy have improved survival of patients into adulthood. Therefore expectation to have a family is an important aspiration for a better quality of life. Although spontaneous fertility can occur in well chelated and transfused patients with spontaneous puberty, majority are infertile due to hypogonadotrophic hypogonadism (HH) and need assisted reproductive techniques (ART). I will report our experience of pregnancy including those following ART in thalassemia patients. A brief outline on prenatal diagnosis will also be addressed and new perspectives of induction of gametogenesis including pregnancy care in patients with thalassemia syndrome will be highlighted. ART in 11 women with β-thalassemia major over the last 15 years at the University College Hospital, London who had HH but functionally intact ovaries will be presented. The major pre-pregnancy issues including pre-pregnancy counseling, partner screening, medications, suitability for induction of ovulation and pregnancy care are reviewed. Pregnancy was advised when patients had echocardiographically normal resting left ventricular performance. Iron overload was assessed by cardiac and hepatic MRI. Cardiac function, along with hematological, endocrine, and hepatic parameters were initially assessed and monitored throughout pregnancy and for 2 to 9 years post partum. All patients were screened for Hep B, Hep C and HIV prior to ART. Partners were investigated for haemoglobinopathy. Desferrioxamine or oral chelators were discontinued. Fourteen healthy newborn infants were delivered. There were 2 sets of twin and one set of triplet pregnancy. One patient developed thrombo-embolic episode, while 2 had pre-eclampsia. The mean serum ferritin concentration increased from pre-pregnancy value of 2000 to 5000 mg/L post delivery. Although no significant cardiac complications were encountered, the incidence of preterm labour and growth restriction were 3 fold higher than the background population. Elective caesarean section was performed in 73% of cases. None of the fetuses had congenital malformations. The mean fetal birth weight was 2.5 kg. Breast-feeding was encouraged in all cases. Chelation therapy was recommenced in the immediate post partum period. In conclusion, pregnancy is feasible in women with β-thalassemia with normal resting cardiac performance and optimized iron overload status. Our experience suggests that, with proper care and guidance, pregnancies in women with thalassemia major are practical and can have successful outcome in specialist centres under a multi disciplinary team.

HETEROGENEITY IN THE CLINICAL SEVERITY OF HBH DISEASE

Vip Viprakasit

Hematology/Oncology Division, Department of Pediatrics and Thalassemia Center, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

Clinical phenotypes associated with α thalassemia result from mutations involving the α globin gene cluster on the telomeric region of the short arm of chromosome 16 (16p 13.3). From two copies of the α globin gene per haploid genome (αα/αα), the α^0 thalassemia (when gene expression is completely abolished), is caused by lost of the both linked α globin genes (--/αα) resulting in mild hypochromic, microcytic anemia in heterozygotes. In α^+ thalassemia, the globin expression is reduced and mainly casued by single α gene deletions (-α) or mutations in one or a few nucleotides in critical regions of the α genes (αTα). Compound heterozygotes for α^0 and α^+ thalassemia results in a severe imbalance in globin chain synthesis giving rise to excess β globin chains precipitate and form a characteristic, Hemoglobin H (Hb H) due to β globin tetramer (β4). Patients with this syndrome have mild to moderate chronic hemolytic anemia and some might be more severe due to additional deleterious effects on terminal erythroid differentiation and red cell metabolism in particular cases with αT mutations. Some might require regular transfusion, however nearly all of them responded well with splenectomy. It is possible that several genetic modifiers might play role on different clinical heterogeneity found in Hb H disease including co-inheritance of β thalassemia, mutations of iron-regulating genes, red cell membrane microskeletal proteins, bilirubin metabolism and erythroid specific transcription factors such as KLF-1 etc. Beside these genetic polymorphisms, variation in clinical severity in Hb H patients could also be contributed by several environmental factors from perinatal and neonatal stress, chronic infection causing active splenic function to organism that directly attacked erythroid progenitor cells such as Parvo-virus B19. Altogether, Hb H disease might not necessarily benign as usually thought and a careful diagnosis by molecular testing of globin defects and others with closely monitoring and follow up in any given patients must be obligatory.

PROMOTION OF HAEMOGLOBINAPATHIES AS A MEDICAL SPECIALTY

Rekha Bajoria, Ratna Chatterjee

Institute For Women's Health, University College Hospital, Department of Obstetrics and Gynaecology, London, UK

Haemoglobinopathies are the commonest global monogenic disorders affecting 420 million worldwide, with 50% childhood deaths and marked morbidity of survivors, the largest in Mediterranean basin, South East Asia and Africa. This is due to poor understanding of disease and lack of resources. There has never been any specialist training for health care professionals caring for these patients. Educating doctors globally is challenging due to widespread dispersal of multidisciplinary experts, disease heterogeneity with cultural diversity. Therefore we have designed a Masters course in Haemoglobinopathy, the first of this kind, in partnership with thalassemia international federation using cutting edge lively state of art media based technology, aiming to attain excellence in teaching and learning within a diverse range of cultural and social setting. The major benefit of this course is that it will allow doctors and other health care professionals to learn about all aspects of Haemoglobinopathy i.e., *Holistic care in Haemoglobinopath*y. The MSc in haemoglobinopathy fulfils all the UCL and TIF's teaching and learning theme of *Education of global citizenship* to address a chronic and a serious disease. The cardinal advantage of the programme is that the doctors can be trained at their workplace and interact with global tutors and students at flexible times in the ideal social and cultural environment through virtual network without brain drain of doctors from the place of work. The extensive use of Internet technology for Web ex tutorials, walk in surgery is key tools for student and tutor engagement. The assessment and maintenance of quality control of the programme are based on strict UCL criteria. We have used course work and on line modular interactive exams as assessment tools which have been validated by stringent UCL criteria. The exit exam is undertaken at British council centres of country of origin of doctors. The course is running successfully for 3 years with very positive feedback from students and National and International tutors. The course Directors have been awarded prestigious UCL Provosts award for excellence in teaching.

COUNTERFEIT/COPY DRUGS: PATIENTS SAFETY IN THE HEART

Yilmaz Capan

Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Ankara, Turkey

Some studies demonstrate that a large proportion of copy drugs contain lower levels of active substances and higher impurity levels than reference drugs. The number of copy drugs failing to meet internationally recognised quality criteria consequences of patients receiving lower doses of active substances than expected. In addition, possible clinical effect of impurities must be considered. Counterfeiting of drugs exhibits a significant and growing threat to human health and public safety. It also results an economic problem for innovator drug manufacturers, undermining their revenues and reputation. Moreover, it negatively affects the confidence of the public in their medicines and the credibility of national health and enforcement authorities. Although precise and detailed data on counterfeit and substandard drugs are impossible to obtain, it is widely assumed that 10% of medicines worldwide are likely to be counterfeit while it is estimated that 5% of all world trade in branded goods is counterfeit, leading to huge financial losses for the pharmaceutical industry. But much more important from public health point of view, is that such products may lead to a great health risk. The essence of counterfeit products and the reason they are so dangerous is the complete absence of quality control, since they are often indistinguishable from the genuine product. Internet-sourced drugs are often considered suspect. The World Health Organization (WHO) reports that drugs from websites that conceal their physical address are counterfeit in over 50 percent of cases; the U.S. Food and Drug Administration (FDA) works with the National Association of Boards of Pharmacy (NABP) to regulary update a list of websites likely to sell drugs that are illegal or of questionable quality.

FROM BENCH TO BEDSIDE: WAYS AND STEPS OF DRUG DISCOVERY

Sefik Alkan

Dept. Mol. Genetics & Microbiology, Johnson Medical School, Piscataway, NJ, USA

Doctors and patients often wonder why drugs are so expensive! It is also difficult to understand why there are only so few new drugs discovered despite higher investments. Even if we know the molecular target it takes too long (10-15 years) to develop a drug and to bring it to the patient. In this presentation, I will guide you through all stages of classical drug development so that we can understand the reasons of the current situation. Although I am not an expert in hematological diseases, the lessons I have learned during my 30 years of experience in the pharmaceutical industry will hopefully allow encouraging discussions to find novel ways for the treatment of thalassemia. Drugs are discovered by many different ways: By following folk medicine (e.g. Aspirin); serendi-

pity (e.g. Desferal); whole animal screening (e.g. cyclosporin); cellular screening (many) and rational design molecular screening (e.g. Gleevec). Today's drug discovery process starts with a *disease hypothesis*, that is, we have to have a good idea about the pathogenesis of a disease. This helps to identify a molecular target. By developing and using an appropriate test, we can screen a huge number of compounds from the chemical library by means of modern robotic systems. First, we find *hits* which may become *leads* for the medicinal chemist. They then optimize the leads and help to select candidate drugs so that preclinical pharmacology can start (early-ADME, toxicology, animal testing etc). If successful, it will enter into the clinic. I will summarize the aims of Phase I-IV clinical trials. The whole drug R&D may take 10-15 years and cost ½ -1 billion dollars. Any drug may *die* at any stage. Thus, drug discovery is a very risky business. I will briefly mention some of the precautions that can prevent costly mistakes. After reviewing the standard treatments for thalassemia, I will summarize the status of new approaches such as stem cell transplants, inserting normal hemoglobin gene into stem cells and triggering patient's fetal hemoglobin.

LIFE STYLE ISSUES IN HAEMOGLOBINOPATHIES

Antonio Piga, Ilaria Salussolia, Simona Roggero

Thalassemia & Haemoglobinopathies Centre, Department of Clinical and Biological Sciences, School of Medicine and Hospital S. Luigi Gonzaga, University of Torino, Italy

Mild forms of haemoglobinopathies do not require special recommendations for life style, whereas for moderate and severe forms the key factor is quality of care. If optimal treatment is available and applied, the patient may enjoy a normal life style and experience regular physical and emotional development. Where the disease cannot be fully compensated with proper care, the patient should be fully informed about the limitations, that are stricter for sickle cell anaemia than for thalassemia. Parents of young patients should be encouraged to balance the attention to prevent negative events (i.e. infections) with a normal social interaction. and during adolescence Depending on the country, thalassemia and sickle cell anaemia may be recognised as disabilities, with resulting benefits and special employment facilities. Care should be taken that these entitlements do not interfere with a positive attitude to normality, self-esteem and the ability to work. Full respect of patient's privacy is the needed premise to let the patient build up a realistic and balanced position between being open and being secretive about the disease. Treating staff should do the best to minimise hospital time. There are no reasons to skip or delay the standard vaccinations or to skip the additional vaccinations recommended for thalassemia and sickle cell anaemia. Special conditions require special attention: i) spleen enlargement requires caution in physical activities and contact sports; ii) splenectomised patient should always travel with antibiotics and have prompt medical attention in case of fever or animal bites; travel where the risk of malaria is significant should be discouraged; iii) carriers of viral hepatitis have to take specific measures to limit the risk of sexual or cohabitation transmission; iv) in conditions like heart disease or osteoporosis, physical activity, if balanced to the clinical condition, is beneficial; v) life style habits are important on long-term evolution of liver disease: alcohol triggers the oxidative damage of iron and aggravates the effect of HBV and HCV on liver tissue. Where all three factors are present, the probability of developing cirrhosis and hepatocarcinoma is significantly raised; vi) alcohol consumption, as well as smoking, are also a facilitator of osteoporosis. Healthy life style habits may significantly improve perspectives of life of haemoglobinopathies and, if perceived *normal*, may improve the quality of life too.

OXIDATIVE STRESS IN THALASSEMIA – POTENTIAL ROLE OF ANTIOXIDANTS

Eliezer Rachmilewitz,[1] Eitan Fidbach[2]

[1]*Edith Wolfson Medical Center, Holon, Israel;*
[2]*Hadassah - Hebrew University Medical Center, Ein-Kerem, Jerusalem, Israel*

Oxidative stress is a major pathophysiological event in patients with homozygous forms of thalassemia. The main cause of oxidative stress is iron overload (IO) resulting from intracellular denaturation of hemoglobin, multiple blood transfusions and increased iron absorption from the G.I.T. One of the consequences of IO is the presence of free iron species – non-transferrin-bound iron (NTBI) and its redox-active form the labile plasma iron (LPI) and the cellular labile iron pool (LIP). These free iron species generate reactive oxygen species (ROS) that cause damage to various cellular components, including DNA, proteins and lipids, in vital organs such as heart, liver and endocrine glands. Hemolysis and ineffective erythropoiesis' resulting in decreased RBC survival and hypercoagulable state also occur. To protect from these deleterious effects, cells have antioxidant systems such as the reduced gluthatione (GSH) that removes ROS by metabolic conversion. Using flow cytometry methodology, oxidative stress parameters (ROS, membrane lipid peroxides, external phosphatidylserine) and free iron species (LIP) were found to be increased with concomitant decrease in GSH – in thalassemic cells compared to their normal counterparts. In view of all these data, the question is how to ameliorate the damage caused by oxidative stress in thalassemia? One target are the free iron species, LPI and LIP, by iron chelators. In addition, specific antioxidants have been used, such as vitamin E, which is a lipid antioxidant that is deficient in homozygous β-thalassemic patients. Another lipid antioxidant and iron chelator is curcumin, a polyphenol-rich herb product used as food additive. Thiol-containing compounds have a profound role by promoting GSH in antioxidant cell defense and redox regulation. Among them are N-acetyl-cysteine and its amide form which is more lipophilic and permeates better RBC as well as blood-brain barrier. Another natural health product is fermented papaya preparation which limits oxidative stress both *in vivo* and *in vitro*. Clinical trials carried out with these antioxidants for up to 6 months clearly demonstrated a significant amelioration of oxidative stress parameters in several forms of homo-

zygous β-thalassemia. However, they were not sufficient to improve hematological parameters. It is possible that longer treatment duration and/or a combination of different antioxidants and iron chelators will yield better results.

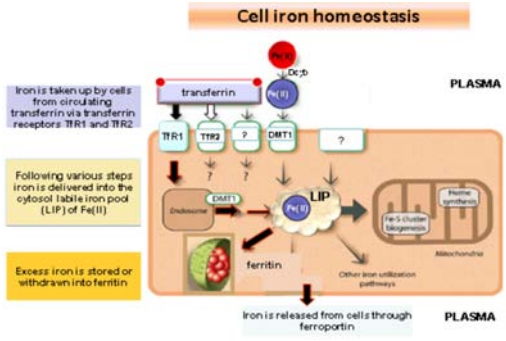


Figure 1.

EXERCISE AND SPORTS IN HEMOGLOBIN DISORDERS

Dilek Yazman

North Cyprus Turkish Thalassemia Center, Turkey

Hemoglobin disorders involve genetic defects in structure or rate of production of globin chains of hemoglobin. The result is anemia and lower oxygen transport to tissues, interfering with normal physical development. Brittle bones which are due to insufficiently treated anemia, osteoporosis, or heavy desferrioxamine use will increase the risk of fractures during many types of exercising, but most importantly with heavy weight lifting or resistance training. Hypoxia will cause muscle pain during exercising and poor muscle and body building. Heart problems, and restrictive type of lung problems which are mostly due to iron loading are major hinderances against exercising capacity, as confirmed in several studies in thalassemia major patients showing pulmonary functional abnormality presenting as restrictive lung disease with increasing age while degree and duration of iron overload was explained as the main factor in its pathogenesis. In recent era with regular blood transfusions and efficient oral or parenteral chelation modalities we see that many patients lead a normal development and life with little or no change from the lifestyle of their unaffected friends. Exercise itself is a measure to improve circulation and oxygenation if enough hemoglobin is present, and hence help growth, muscle and bone building. Smoking cessation, good nutrition with enough protein, calorie, calcium, vitamin D and exercising on a regular basis are important points for maintaining healthy growth and development. Exercise will help prevent fractures and bone deformities. For strong bones, brisk walking, jogging, running, aerobics, step, dancing, circuit training, weight bearing exercises will be suggested according to the person's medical condition. There are cognitive and emotional factors leading behaviour for participation in the social life and exercise, as studied by many scientists in Italy, United Kingdom, Greece and India. Most of them were carried out with interviews using different schedules. In 2006, the Indian researchers found that adverse impact of thalassemia was mainly perceived in education (70%) and sports (72%). The study population confessed physical weakness as hindering participation in the physical activities, but also admitted disappointment for their physical appearances as a negative self concept.

CHOOSING THE RIGHT DIAGNOSTIC APPROACH

Mary Petrou

Haemoglobinopathy Genetics Centre, University College London, Institute of Women's Health, University College London Hospitals, NHS Foundation Trust, London, UK

The beginning of the second decade of the new millennium seems a good time to reflect on the enormous changes that have taken place in the field of genomics. The Human Genome Project, an important feature in the molecular revolution involved an international effort to create an ordered map of the human genome and make it available worldwide. The HGP was completed in 2003 and the detailed knowledge of the human genome provides new avenues for advances in medicine and biotechnology. These advances will touch the lives of many but what is the fate for low resource countries? There were already existing genomic advances that were known before the start of the human genome project. Such is the case for thalassemia. We are unlikely to learn more about any disease than we already know about thalassemia. We know the gene and its mutations, the mechanism of loss of function, yet we do little for the majority of the patients. We know the mutations that cause thalassemia, there is reliable technology for carrier detection and molecular technology for prenatal diagnosis but it is little used in many countries where the incidence of thalassemia is high and the populations are large. Or where used in these countries, it has little effect in reducing the huge number of affected children born. The HGP invested large sums of funds to address the ethical, legal and social issues (ELSI) that may arise from the project. However major ELSI issues have not been addressed, such as the inability of the knowledge acquired to be translated for the benefit of developing countries, how to provide optimum services to patients and how the existing technology can be used to provide prevention programmes for countries such as India, Pakistan, Bangladesh, Central Asia, South East Asia, countries where the incidence of thalassemia is significant. The ultimate goal; to provide a truly informed choice for couples at risk for thalassemia disorders. Which are the right strategies and diagnostic methods for these countries? Can we employ suitable strategies for these countries that will provide reliable results and reduce the burden for *at risk couples*?

DETECTION OF COMPLEX HEMOGLOBINOPATHIES – RECOMMENDATIONS ON SCREENING AND DNA TESTING

Erol Baysal

Dept. of Pathology & Genetics, Dubai Genetic & Thalassemia Center, Dubai, United Arab Emirates

Common hemoglobinopathies such as β-thal, α-thal, HPFH, δβ-thal and abnormal Hbs (HbE, S, C, Lepore, D-Punjab, O-Arab) can be detected easily. Their accurate identification can provide valuable diagnostic and prognostic options for clinicians and for couples who may have to consider family planning. The diagnosis of hemoglobinopathies has become an increasing challenge in the multinational countries such as Australia, USA, Canada as well as in Europe and the Gulf Countries. This is well exemplified by Dubai, which is home to 202 different nationalities (latest figures), hence the propensity for significantly enriched thalassemia gene pool coupled with high degree of consanguinity. In an attempt to curb the hemoglobinopathy problem, the National Premarital Screening Program was rendered mandatory in Dubai in 2006 for all nationalities and the Prenatal Diagnosis Program has been underway successfully since 2005. These preventive programs were imperative as hemoglobinopathies are a major public health concern in the UAE. Dubai is arguably the most heterogeneous hemoglobinopathy nation in the world with 55 β-globin gene defects reported to date. It is anticipated that various complex hemoglobinopathies with extensive heterogeneity in genotype and variable phenotype will emerge from such admixture of genes in a small nation where first cousin marriage among the indigenous population is a norm and not an exception. In addition, α-thal and β-thal interactions occur due to relatively high frequencies of α globin and β globin gene defects; 50% and 8.3%, respectively. This makes clinical diagnosis and laboratory evaluation much more challenging in a young nation where 26% of the population is below age 15 and 71% is between 15-64. The laboratory diagnoses of hemoglobinopathies are often made with certain assumptions: i) variation in the phenotype could be a reflection of interplay between different abnormal globin genes (α, β, γ, δ); ii) same genotypes may have different phenotypes in different geographical areas thus denoting the role of environment as a modulator; iii) complex genotypes occur rarely so no concrete conclusions must be drawn from only a few examples. Here we provide guidance to clinicians, counsellors and healthcare providers on complex hemoglobinopathies only. Several real case scenarios and various algorithms will be discussed to reach correct diagnoses. Comparison from different laboratories will be presented in order to highlight the importance of appropriate lab assessment and interpretation especially with reference to different normal ranges for MCV, MCH, HbA2 adopted in different labs.

STATE-OF-THE-ART HEMOGLOBINOPATHY DIAGNOSTICS. IMPLEMENTING DIAGNOSIS, PREVENTION AND RESEARCH IN THE NETHERLANDS

Cornelis Harteveld

Dept of Human and Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands

The disease. Hemoglobinopathies (HbP) are the most common monogenic recessive trait in man. The disorders are caused by mutations on the globin genes that may change the structure of the gene products (abnormal hemoglobin's) or that may impair the expression of the genes (thalassemia's). Because the trait (carrier state) have protected many world populations during the last 10.000 years against death in infancy due to *malaria tropica*, at least 7% of the world population originating from the tropical and subtropical belt of the old world is today a (healthy) HbP carrier. Children of parents who are both healthy carriers have 25% chance of being severely affected with the most common severe forms i.e. sickle cell disease (SCD) and β-thalassemia Major (Cooley anemia).[1] *The proteins.* Sickle cell disease and β-thalassemia major are disorders involving a protein essential for life, the hemoglobin molecule. The HbA tetramer (α2/β2) is the protein that transports oxygen trough our body. HbA is the major component of the red cells in postnatal life and like the red cells has a lifespan of 120 days. During an average lifespan we need to make 300 kg of hemoglobin and for this we need several perfectly functioning globin genes. *Genetics*. In human α- and β-like globins are coded by two gene clusters located on chromosomes 16 and 11 respectively. Embryonic genes (ζ_2 and ε) are only active during early embryonic life, producing Hb Gower-1, Gower-2 and Hb Portland. Two α-genes per chromosome (two of maternal and two of paternal origin, 4 in total = αα/αα) are expressed throughout fetal life producing the α globin needed for the formation of fetal hemoglobin (HbF = γ2/α2) in combination with the γ chains coded by 4 γ genes per chromosome (also two of maternal and two of paternal origin). Shortly after birth the 4 genes lose their expression while 2 β genes (one of maternal and one of paternal origin) become active. The 4 α genes stay active in postnatal life coding for the same α globin chains, now needed for the formation of adult hemoglobin HbA (α2/β2). Equally, the 4 α genes, with a limited amount of δ chains expressed by the 2 δ genes, also contribute to the formation of the minor postnatal tetramer HbA_2 (α2/δ2). This Hb fraction has no pathological relevance but is of significant interest for the diagnosis of β-thalassemia carriers. Keeping in mind the number of genes involved at a specific stage of life is important for the interpretation of the laboratory results. Because of the pre- and post-natal expression, pathological genotypes involving the 4 α genes manifest both in pre- and postnatal life while pathological β-globin genes are only significantly expressed in post-natal life. Therefore, sickle cell disease and β-thalassemia major come to expression only in the after-birth stages of life, when non-affected fetal cells have disappeared.[2] *Basic diagnostic tools for the hematology lab*. The tools for basic diagnostics are still based on the basic hematologi-

cal parameters and on the separation and estimation of the hemoglobin fractions. Dedicated HPLC and CE devices available on the market recognize all abnormal separations at a high degree of sensitivity. Frequent traits can be confirmed by simple or more complex additional analysis, however, although very sophisticated, HPLC and CE present, like any analytical method with inevitable limitations. A normal individual after the age of 2 will present with about 96-97% HbA, ±3% HbA_2 and <1%HbF. Any change in this pattern will be anomalous might indicate an hemoglobinopathy and will have to be investigated. In the mixed Dutch population the prevalent HbP traits are HbS (the cause of sickle cell disease), β- and α-thalassemia. HbS, very common in the Netherlands, will be easily detected as an anomalous fraction of about 40% or less in the healthy carrier and of about 80-90% in not jet transfused sickle cell patients. Equally, new born with the trait or the disease will present with ±80% HbF and 10% HbA and 10% HbS or with ±20% HbS only, respectively. Carriers of β-thalassemia will be diagnosed from the age of 1 by their microcytic hypochromic anemic state and by the elevated level of the HbA_2 fraction (>4%, N= 2.5-3.5%). The child affected with β-halassemia major will be severely anemic and not able to produce the normal HbA fraction. If not yet transfused, they will present with HbF and HbA_2 only. α^+-thalassemia is very common in the world population and can only be suspected at the hematological and biochemical level due to microcytic hypochromic parameters and eventually a reduced HbA_2 level. Confirmation of α-thalassemia is done at the molecular level. *Molecular diagnostics.* Molecular analysis is needed to confirm and for the prognosis of β-thalassemia and to offer prevention by early pregnancy molecular diagnostics. The main technologies involved are direct DNA sequencing for the many β-thalassemia and α-thalassemia point mutations and for SCD. Gap-PCR is the technology of choice for the common α-thalassemia deletions and MLPA is the ultimate solution for unknown deletion defects of either the β- or α-globin genes cluster. The most established approach in DNA diagnostics to screen for the most common deletion defects causing α-thalassemia or β-thalassemia is gap-PCR, because the method makes use of equipment already available in most diagnostic laboratories, is inexpensive and fast. For less common rearrangements in the α- and β-globin gene clusters, Multiplex Ligation-dependent Probe Amplification (MLPA) has become a standard tool,[3] besides Southern blotting and cytogenetic methods. MLPA is a technology based on ligation of probe-pairs hybridized to a region of interest to detect deletions or duplications by quantitative PCR and fragment analysis. Due to the implementation of MLPA for the detection of copy number variation in the α- and β-globin gene clusters, more and more new types of deletions were detected. But also the discovery of duplications of the complete α-globin gene cluster including the Major Conserved Regions shed light on some unexpectedly severe cases of β-thalassemia intermedia in β-thalassemia carriers. However, by using MLPA the exact breakpoints of these novel deletions and duplications remain unknown. Knowledge of breakpoint sequences might give more insight in the molecular mechanisms giving rise to these rearrangements and may facilitate primer design for gap-PCR to screen for certain common population specific deletions.[4] For this purpose a custom fine-tiling array for high resolution breakpoint determination was designed to perform array Comparative Genome Hybridization. The oligonucleotides cover the complete α- and β-globin gene clusters including the neighboring regions. Based on the results breakpoint primers were designed to perform gap-PCR and breakpoint sequencing.

References

1. Steinberg MH et al. Disorders of Hemoglobin. 2001 Cambridge University Press.
2. Giordano PC, Harteveld CL. Prevention of hereditary haemoglobinopathies in The Netherlands. Ned Tijdschr Geneeskd 2006;150:2137-41
3. Harteveld CL, Voskamp A, Phylipsen M, Akkermans N, den Dunnen JT, White SJ, et al. Nine unknown rearrangements in 16p13.3 and 11p15.4 causing alpha- and beta-thalassaemia characterised by high resolution multiplex ligation-dependent probe amplification. J Med Genet 2005;42:922-31.
4. Harteveld CL, Refaldi C, Cassinerio E, Cappellini MD, Giordano PC. Segmental duplications involving the alpha-globin gene cluster are causing beta-thalassemia intermedia phenotypes in beta-thalassemia heterozygous patients. Blood Cells Mol Dis 2008;40:312-6. Epub 2008 Jan 14.

INTERACTOMICS APPROACH IN HEMOGLOBINOPATHIES

Erol Ömer Atalay

Pamukkale University Medical Faculty Department of Biophysics, Denizli, Turkey

Protein-protein interactions are major elements in living cells either in normal and pathological states. Interactions between these proteins have central roles in all cellular processes under interest. Up to date technologies based on bioinformatics and novel biophysical techniques at molecular level cause many developments in understanding the molecular processes involved during normal and pathological states leading to the development of novel therapeutic and diagnostic strategies. Determining the entire human genome sequences was the first step in genomics research. During genomics era, new molecular techniques including genome sequencing, based on PCR (polymerase chain reaction) were developed and huge amount of genomic data were collected. The study of human genome identified the individual characteristics of the human genome and collected enormous amount of data on human genetic diversity. In the post genomic era, novel omics approaches has become under interest by the researchers. The advent of these novel approaches has been directed to the large number of transcripts (transcriptomics), DNA and chromation modifications (epigenomics), metabolites (metabolomics), degradation processes (degradomics), interactions proteins (interactomics) etc. Phage and peptide display approaches are being used to identify the interacting proteins and peptides against the targets under interest. Developed peptides based on display techniques can be used as functional molecules in possible therapeutic strategies and also as specific biological sensing diagnostic tool. As it is well known, hemoglobinopathies are genetically based blood

disease commonly seen in world populations. In the genomics era, many diagnostic tools were developed to detect the molecular problem of these blood diseases. In the post genomic era, the therapeutic and diagnostic problems in the hemoglobinopathies need to be solved due to the lack of scientific information in protein-protein interactions leading to the development of the normal and pathologic disease states. The potential interactomics targets in hemoglobinopathy research are the red cell membranes and hemoglobins under interest. Here in this symposium; the results of functional peptides against living cells (K562 cell line) and peptides recognizing Hb A2 and Hb S will be presented. According to the results obtained, the use of peptide libraries against living cells and abnormal hemoglobins will be discussed.

Index of authors