



October 21-24, 2009

Ninth Cooley's Anemia Symposium

**ABSTRACTS FOR POSTER SESSIONS & DATA-BLITZ
PRESENTATIONS**

POSTER PRESENTATIONS

Posters are scheduled to be exhibited on **Thursday, October 22nd** and **Friday, October 23rd**
Times for set-up of poster displays are indicated in the Final Program.

SCIENTIFIC ORGANIZING COMMITTEE

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Data Blitz Presenters:

Friday, October 23, 2009

1. CARDIAC RON LOADING MEASURED BY MULTISLICE MULTIECHO T2* CMR AND CARDIAC DISEASE IN MALE AND FEMALE PATIENTS WITH THALASSEMIA MAJOR

Caterina Borgna-Pignatti, MD¹, Antonella Meloni, MSc,² Valeria Caldarelli, MD¹, Nicolò Zanforlin MD¹, Maria Marsella, MD, Chiara Dell'Amico, MSc,² Anna Spasiano, MD,³ Lorella Pitrolo, MD,⁴ Eliana Cracolici, MD,⁵ Gianluca Valeri, MD,⁶ Massimo Lombardi, MD,² Alessia Pepe, MD, PhD²

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Female patients with thalassemia major survive longer than males and the difference is due to a lower rate of cardiac disease in females. As a part of the MIOT project we studied 776 consecutive patients (370 males) using the multislice multiecho T2* CMR technique to quantify segmental and global myocardial iron overload in males and females. The MIOT project is a previously validated network of 6 CMR and 56 thalassemia centers sharing a clinical and instrumental database. 43 males and 34 females (difference not significant) had EF<50% or had a positive history of cardiac failure requiring therapy. The risk of having cardiac failure increased with age. Cardiac T2* was significantly lower in patients with cardiac failure (p=0,001), but no sex difference was observed. 31 males and 12 females developed cardiac arrhythmias. Their cardiac T2* was significantly lower than that of patients without arrhythmias (24±15 vs 29±12 ms; P=0.033 in males and 19±12 vs 28±13; P=0.018 in females). Liver T2* was similar in male and female patients with or without heart disease. Ferritin levels were significantly higher in male patients with heart disease, but again they were not different in males and females. We conclude that males and females are at the same risk of accumulating iron in their hearts, but that females tolerate iron toxicity better, possibly as an effect of reduced sensitivity to chronic oxidative stress.

2. HJV IS NOT REQUIRED FOR HEPCIDIN EXPRESSION IN ZEBRAFISH EMBRYOS

Yann Gibert¹, Victoria J. Lattanzi¹, Jodie Babitt², Herbert Y. Lin², Matthias Hammerschmidt³, Paula G. Fraenkel¹

¹Division of Hematology/Oncology, Beth Israel Deaconess Medical Center and Harvard Medical School, ²Program in Membrane Biology and Nephrology Division, Massachusetts General Hospital and Harvard Medical School, ³Max-Planck Institute for Immunobiology, Freiburg, Germany

The leading cause of death in Cooley's anemia is heart failure related to chronic iron overload. Heparin is a transcriptionally regulated peptide hormone, produced by hepatocytes in response to iron overload, which decreases intestinal iron absorption. Heparin levels are inappropriately low in patients with Cooley's anemia. We have been developing the zebrafish embryo as a model to identify *hepcidin* regulators, which may be exploited to prevent and treat iron overload in Cooley's anemia. Recently, it has been shown in mammalian models that membrane-bound *hemojuvelin* (*hju*) acts via the bone morphogenic protein (BMP) signaling pathway to stimulate *hepcidin* expression. *Neogenin* and *furin* have also been implicated in *hju*'s function. Previously, we demonstrated that *hepcidin* expression in zebrafish embryos increases in response to iron loading and requires the zebrafish orthologs of *transferrin* and *transferrin receptor*. We hypothesized that the BMP signaling pathway and *hju* would regulate *hepcidin* expression in zebrafish embryos. We used whole mount *in situ* hybridization, quantitative realtime PCR, morpholino knockdowns and overexpression experiments to study the expression and function of *hju*. We found that overexpression of BMP2b increases *hepcidin* transcript levels, but the BMP inhibitor, dorsomorphin, abrogates them. We demonstrated that *hju* is strongly and sequentially expressed in the notochord and somites and that knockdown of *hju* resulted in severe defects in these structures. At the time of *hepcidin* expression, however, *hju* was not detectable by *in situ* hybridization in the liver of the zebrafish embryo. Knockdown of *hju* failed to abrogate *hepcidin* expression, although it did impair liver development. Furthermore, knockdown of *hju* failed to prevent the upregulation of *hepcidin* expression caused by overexpression of *BMP2b*. We found that morpholino knockdown of the zebrafish orthologs of *neogenin* or *furin* also failed to affect *hepcidin* expression. Taken together, these data indicate that activation of the BMP signaling pathway induces *hepcidin* expression in an *hju*-independent manner in zebrafish embryos. This differs from post-natal mice in which *hju*-deficiency results in severe *hepcidin* deficiency. We conclude that the zebrafish embryo provides insight into *hju*-independent mechanisms of *hepcidin* regulation.

3. BLOOD SAFETY SURVEILLANCE IN THALASSEMIA PATIENTS

Sean Trimble, MPH, Nimia Reyes, MD, Althea Grant, PhD, Centers for Disease Control and Prevention, Atlanta, Georgia, Thalassaemia Treatment Center Coordinating Committee

CDC established the Thalassaemia Data and Blood Specimen Collection System (TDC) for the early detection of known and emerging infections that might be transmitted through blood transfusion. Through a cooperative agreement, CDC provides funding to 7 Thalassaemia Treatment Centers (TTCs). The TTCs participate in CDC's blood safety surveillance program by sending blood specimens of consented patients to CDC annually to be screened for HIV, hepatitis A, B, and C, and other emerging blood-borne pathogens; this allows the identification of patients who may have become infected as a result of contaminated blood products. A specimen repository of tested blood samples has been established at CDC that could be utilized for research or investigations of blood-borne emerging pathogens.

Staff from each Thalassaemia Treatment Center (TTC) obtained informed consent from each TDC participant to collect a uniform set of clinical data and a blood specimen to serve as a baseline upon enrollment. Additional clinical data and blood specimens are obtained at each annual visit. The TDC program has 408 patients enrolled. Since specimens began to be collected in 2004 until the end of 2008, we have collected 1036 blood samples. There have been a total of 78 seroconversion alerts that have been investigated. Of these seroconversion alerts, 64 have been resolved (49 hepatitis A, 11 hepatitis B, 4 hepatitis C) based on the findings of the investigations. Of the resolved cases, 15 were resolved as false positives, 7 were identified previous infections, and 42 were identified as vaccination responses. There are currently 14 open, on-going investigations requiring additional data to confirm or rule-out viral hepatitis seroconversions. There have been no HIV seroconversion alerts to date.

Our results provide support for the use of special efforts in the surveillance of Thalassaemia patients. Transfusions are very prevalent in this population and sentinel surveillance could be used to monitor the safety of the national blood supply. Such data can also be utilized for analyzing vaccination trends.

4. CLINICAL TRIALS IN THALASSEMIA: INSIGHTS FROM THE PATIENT COMMUNITY

Gina Cioffi, JD, Eileen Scott Cooley's Anemia Foundation, New York, NY

Background: Among people with severe chronic illness only 6% participate in clinical trials. Objectives: A survey was conducted to assess the participation of US thalassaemia patients in clinical trials and to discern their level of awareness, access and trust in clinical trials. Methods: 25 patients were asked to take a 17-question survey regarding clinical trials offered over the past ten years. Results: Awareness of trials was high and generally favorable. 84% of those surveyed were approached to participate in a trial. 67% are currently enrolled in a clinical study. Access to trials was also high. 76% met eligibility for a trial. 60% were approached for trials they subsequently did not qualify for and 87% would have participated if they met the criteria. Trust in clinical trials varied. 80% felt they were adequately informed about a specific trial and over 90% believe that the trials offered over the past ten years were vital and ultimately will lead to a better quality of life. However, only 12% of patients believe that clinical trials are very safe. Patients provided feedback on how trials and their importance can be better communicated and conducted. They strongly believe (80%) that the trials they were aware of were not designed to accommodate their personal needs. Only 4% of patients had been approached by an investigator regarding their opinions on the design of a clinical trial. Conclusion: Recruitment may increase if patient feedback was integral to study design, time constraints of working adults need consideration, patients desire follow-up on trial results.

Saturday, October 24, 2009

1. THE HUMAN ANKYRIN REGULATORY ELEMENT INCREASES THE EXPRESSION OF THE TRANSGENIC HUMAN B-GLOBIN GENE

Laura Breda¹, PhD, Carla Casu¹, B.Sc., Laura Casula¹, PhD, Lawrence B. Gardner², MD, Stefano Rivella¹, PhD ¹Weill Cornell Medical College, ²New York University School of Medicine, New York, NY

We observed that a lentiviral vector, carrying the human β -globin gene (T9W, see Breda #1), failed to increase hemoglobin A (HbA) in cells of patients carrying alternative splicing mutations, in both alleles, of the β -globin gene. HbA did not increase, even though the transgenic β -globin mRNA was expressed at high level and the presence of alpha-globin aggregates was observed. We observed that these aberrant mRNAs generated by the alternative splicing were stable and, for this reason, we hypothesized that they compete with and/or prevent the translation of transgenic β -globin mRNA. We generated a second lentiviral vector in which a human ankyrin regulatory element flanks the transgenic human β -globin cassette (AnkT9W), after chromosomal integration. We compared the two lentiviral constructs in Murine Erythro Leukemia (MEL) cells. Compared to T9W, AnkT9W expressed up to 3.5 times more human β -globin mRNA and produced nearly two times more absolute chimeric hemoglobin (α -mouse: β -human). Furthermore, AnkT9W-RNA fractions in the cytoplasm had a net shift toward the highest multi-polysomal component, implying a translational advantage. In mice, AnkT9W markedly improved the phenotype of thalassemic animals engrafted with lentiviral transduced thalassemic bone marrow cells. And finally, AnkT9W increased the HbA in cells of patients carrying alternative splicing mutations, simultaneously reducing the amount of alpha-aggregates (see Breda #1). In order to investigate whether the ankyrin element is responsible for epigenetic modifications of the beta-globin cassette and whether the ankyrin either acts as an insulator or enhancer element, we are performing chromatin analyses and generating new vectors to characterize its function.

2. OVEREXPRESSION OF HAMP TO REDUCE IRON OVERLOAD AND IMPROVE ANEMIA IN THALASSEMIC MICE

Sara Gardenghi¹, Maria Franca Marongiu¹, Ella C. Guy¹, Kristen Muirhead¹, Cindy N. Roy², Nancy C. Andrews³, Eliezer A Rachmilewitz⁴, Patricia J. Giardina¹, Robert W. Grady¹ and Stefano Rivella¹ – ¹Weill Cornell Medical College, New York, NY; ²Johns Hopkins University School of Medicine, Baltimore, MD; ³Duke University School of Medicine, Durham, NC; ⁴Hematology Department Edith Wolfson Medical Center, Holon, Israel

The hepatic peptide hepcidin (*Hamp*) negatively regulates iron absorption degrading the iron exporter ferroportin at the level of hepatocytes and macrophages. Mice with beta-thalassemia intermedia (*th3/+*) show progressive iron overload, while *Hamp* is expressed at low level. We hypothesized that *th3/+* mice absorb more iron than required for erythropoiesis and that increasing *Hamp* levels could limit their iron intake without impairing macrophages function and erythropoiesis. As a control, we fed wild-type (wt) and *th3/+* mice with diets containing 2.5- and 35-ppm iron (low iron and iron sufficient, respectively) and compared them to wt and *th3/+* transgenic mice over expressing *Hamp* (*Tg-Hamp* and *Tg-Hamp/th3*) fed the 35-ppm diet. We analyzed mice hematological parameters, organs iron content, and *Hamp* levels after 1 and 5 months. Compared to the 35-ppm, the 2.5-ppm iron diet produced anemia and reduced tissue iron content and *Hamp* levels in wt mice. However, although the 2.5-ppm diet decreased the liver and spleen iron content and *Hamp* levels also in *th3/+* mice, it did not make them more anemic. In *Tg-Hamp/th3* mice the iron content of the liver and spleen was reduced to almost normal levels. However, while *Tg-Hamp* mice showed anemia, the hematological parameters and splenomegaly of *Tg-Hamp/th3* mice improved significantly. In conclusion our data indicate that overexpression of *Hamp* could reduce tissue iron overload with beneficial effects on erythropoiesis and splenomegaly.

3. USE OF JAK2 INHIBITORS TO LIMIT INEFFECTIVE ERYTHROPOIESIS AND IRON ABSORPTION IN A MOUSE MODEL OF BETA-THALASSEMIA

Luca Melchiori, BS¹, Sara Gardenghi, PhD¹, Ella Guy, BS¹, Eliezer Rachmilewitz, MD², Patricia J Giardina, MD¹, Robert W Grady, PhD¹ and Stefano Rivella, PhD¹, ¹Weill Cornell Medical College, New York, New York, ²Wolfson Medical Center, Israel

Beta-thalassemia intermedia and major are characterized by Ineffective Erythropoiesis (IE), with complications such as splenomegaly and iron overload due to decreased expression of hepcidin. Using a mouse model of beta-thalassemia major (*th3/th3*) and intermedia (*th3/+*) we showed that the Jak2 kinase plays a pivotal role in IE and that the administration

of a Jak2 inhibitor reverts splenomegaly but also affect anemia. From these findings we formulated two hypotheses. 1) Since the majority of thalassemic patients are transfused, the administration of a Jak2 inhibitor together with blood transfusions might reduce the splenomegaly without affecting the anemia and 2) the administration of a tailored dose of the drug to untransfused animals could decrease splenomegaly and iron absorption without affecting anemia. Compared to the placebo treated transfused group, *th3/th3* mice transfused and treated with the drug showed reduced spleen size and extramedullary erythropoiesis, with increased levels of hemoglobin, probably due to the spleen reduction. *Th3/+* mice not transfused and treated with a tailored dose of the drug not only showed decreased spleen size, but their hemoglobin levels were not reduced compared to the control group. For both transfused *th3/th3* and not transfused *th3/+* mice treated with the drug the expression of hepcidin was increased and correlated with the spleen weight. These results support our hypotheses and show that the use of Jak2 inhibitors could be a novel therapeutic approach to target IE and ameliorate the iron overload in Thalassemia.

POSTER PRESENTERS – Thursday, October 22

1. Osheiza Abdulmalik
2. Laura Breda
3. M Domenica Cappellini
4. Amrita Dutta
5. Elalfy Mohsen
6. Elalfy Mohsen
7. Giuliana Ferrari
8. Gian Luca Forni
9. Suthat Fucharoen
10. Suthat Fucharoen
11. Suthat Fucharoen
12. Yelena Ginzburg
13. Kallistheni Farmaki
14. Kallistheni Farmaki
15. Inderdeep Kalra
16. Maria Eliana Lai
17. Guyu Liu
18. Rashid Merchant
19. Leila J. Noetzli
20. Inusha Panigrahi
21. Nagina Parmar
22. John B. Porter
23. Geetha Puthenveetil
24. Pedro Ramos
25. Sally Yu Shi
26. Suthat Fucharoen
27. Saovaros Svasti
28. Saovaros Svasti
29. Vip Viprakasit
30. Vip Viprakasit
31. Vip Viprakasit
32. Maria Vogiatzi
33. Bindu Kanthezhath
34. Patrick B Walter
35. Giovanna Graziadei

1. STRUCTURAL AND FUNCTIONAL ANALYSES OF HYPERSTABLE β -GLOBIN MRNAS

Osheiza Abdulmalik, DVM¹ and J. Eric Russell, MD^{1,2}. ¹Hematology, Children's Hospital of Philadelphia, and ²Medicine (Heme-Onc), University of Pennsylvania, Philadelphia, PA

We previously proposed that the efficacy of therapeutic transgenes encoding human β -globin could be improved by structural modifications that prolonged the cytoplasmic half-life of its cognate mRNA. Previous analyses have linked the constitutively high stability of β -globin mRNA to a region of its 3'UTR that is predicted to assemble an extended stem-loop (SL) structure. We reasoned that duplication of the SL motif might prolong the survival of β -globin mRNA, resulting in higher levels of both β -globin mRNA and its encoded protein. Using an enzymatic mRNA secondary-structure mapping strategy, we demonstrated the assembly of the predicted SL motif in the β -globin 3'UTR. To test whether SL duplication could stabilize β -globin mRNA *in vivo*, we constructed full-length β -globin genes--linked to tetracycline-suppressible promoters--containing 3'UTRs modified to contain either two or three tandem SL motifs. Transiently-transfected K562 cells expressing the SL-variant and control wild-type β -globin genes were exposed to tetracycline, and levels of β -globin mRNA determined by qRT-PCR at defined intervals using tet-indifferent β -actin mRNA as internal control. Relative to wild-type β -globin mRNA, SL-duplicate β -globin mRNAs displayed a position-dependent 2-fold increase in cytoplasmic half-life; SL-triplicate β -globin mRNAs did not exhibit any additional stability. To investigate the mechanistic basis for mRNA hyperstability, we constructed erythroid K562 and non-erythroid HeLa cells to stably express both wild-type and SL-duplicate β -globin mRNAs. Preliminary experiments validate the beneficial effects of SL duplication on the half-life of β -globin mRNA. mRNA cloning analyses reveal a previously unknown heterogeneity in the position of 3' polyadenylation in both wild-type and SL-duplicate β -globin mRNAs; corresponding RNase H-based analyses of poly(A) tail lengths are currently in progress. These experiments confirm the existence of a predicted SL structure within the β -globin 3'UTR, and demonstrate that duplication of this motif can substantially increase the stability of β -globin mRNA. Results from this investigation will be immediately applicable to the development of high-efficiency therapeutic transgenes for thalassemia.

2. FOLLOWING B-GLOBIN GENE TRANSFER, THE PRODUCTION OF HEMOGLOBIN DEPENDS UPON THE B-THALASSEMIA GENOTYPE

Laura Breda, PhD¹, Carla Casu, B.Sc.¹, Dorothy A Kleinert, N.P.¹, Luca Cartegni, PhD², Eitan Fibach, PhD⁴, Roberto Gambari, PhD³, Patricia J Giardina, MD¹ and Stefano Rivella, PhD¹. ¹Weill Cornell Medical College; ²Memorial Sloan Kettering Cancer Center, New York; ³University of Ferrara, Italy and ⁴Hadassah University Hospital, Jerusalem, Israel

B-thalassemia mice can be rescued by lentiviral-mediated β -globin gene transfer. Based on these and other preclinical studies, clinical trials are underway. To date, however, no study has addressed the efficacy of gene therapy in relationship to the different nature of the β -globin mutations. All mutations can be classified as β^0 or β^+ , in which none or insufficient β -globin protein is produced, respectively. We amplified and differentiated *in vitro* erythroid progenitor cells (ErPCs) isolated from peripheral blood mononuclear cells of β -thalassemia patients (N=33). Cells were infected with either T9W or AnkT9W, lentiviral vectors carrying the human β -globin gene, the human LCR and, in AnkT9W, a novel genomic element associated with the human ankyrin locus. Using T9W, ErPCs of β^0/β^0 patients presented HbA levels increasing proportionally to the number of genomic integrations per cell and reaching values similar to the expected normal ones (N=10). Conversely, β^+/β^+ cells responded only slightly or not at all. In β^0/β^+ cells we observed mixed results. We hypothesized that the aberrant mRNAs made in β^+/β^+ cells prevent the transgenic β -globin mRNA from being translated. The AnkT9W vector completely rescued Hb synthesis in β^+/β^+ cells (N=5), with HbA increasing in a vector-copy-number dependent manner. We believe that the ankyrin element is responsible for increasing the transcription of the β -globin transgenic mRNA during erythroid differentiation, efficiently competing with the aberrant β^+/β^+ mRNA for translation (see Breda #2). These new findings may have profound implications in designing gene therapy trials and in understanding the genotype/phenotype variability observed in Cooley's anemia.

3. UP TO 4.5 YEARS OF DEFERASIROX (EXJADE®) TREATMENT IN PATIENTS WITH THALASSEMIA MAJOR: A POOLED EFFICACY AND SAFETY ANALYSIS

M Domenica Cappellini, MD¹, Renzo Galanello, MD², Antonio Piga, MD³, Antonis Kattamis, MD⁴, Yesim Aydinok, MD⁵, Darlene Lagrone⁶, Victor Dong, MD⁷, John B Porter, MA., MD⁸, ¹Università di Milano, Italy, ²Università di Cagliari, Italy, ³Università di Torino, Italy, ⁴University of Athens, Greece, ⁵Ege University Medical Faculty, Turkey, ⁶Novartis Pharmaceuticals, USA, ⁷Novartis, Switzerland, ⁸University College London, UK

The long-term efficacy and safety of deferasirox (Exjade®), a once-daily oral iron chelator, was evaluated in a pooled analysis of four 1-year core and subsequent extension trials (105E–108E) in adult and pediatric iron-overloaded patients

with β -thalassemia. Initial deferasirox doses were assigned by baseline liver iron concentration, with subsequent dose adjustments during the extension phases based on serum ferritin (SF) levels. Efficacy was evaluated by monthly SF measurement and safety by adverse events (AEs) and laboratory parameters. This analysis included 472 patients treated with deferasirox at an overall mean \pm SD daily dose of 22.1 ± 6.4 mg/kg/day, increasing from ~ 19.5 at 1 year to 25.2 mg/kg/day by 4.5 years. By 4.5 years, 106 patients were receiving <20 mg/kg/day, 193 were receiving 20 – <30 mg/kg/day and 168 were receiving ≥ 30 mg/kg/day. Mean iron intake over the entire treatment period was 0.4 ± 0.1 mg/kg/day. Median SF was 2319 ng/mL at baseline; change from baseline was 158 ng/mL at 1 year, and -931 ng/mL by 4.5 years ($P < 0.0001$). Treatment was discontinued in 159 patients, for reasons including consent withdrawal (11%), AEs (11%), and unsatisfactory therapeutic effect (8%). All five deaths were considered unrelated to deferasirox (sepsis [$n=1$] and cardiac failure [$n=4$]). The most common drug-related AEs were abdominal pain (13%), nausea (12%), diarrhea (9%), vomiting (7%), and rash (5%), with frequency decreasing year by year. Non-progressive increases in serum creatinine and alanine aminotransferase occurred in 7% and 6% of patients, respectively. In conclusion, deferasirox achieved a reduction in SF in patients with β -thalassemia treated for up to 4.5 years, and was generally well tolerated.

4. MOLECULAR MECHANISMS OF EMBRYONIC/FETAL GLOBIN GENE INDUCTION BY SHORT-CHAIN FATTY ACIDS: INSIGHTS FROM A NEW MODEL

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Increased expression of fetal hemoglobin (HbF) can ameliorate β -thalassemia. Short chain fatty acids (SCFAs) can increase HbF *in vitro* and *in vivo*, although the molecular mechanisms through which this is accomplished are not well understood. Limited mechanistic insights are likely partly attributable to the small number of *ex vivo* models. We have overcome some limitations of previously used models by establishing a primary cell model of SCFA-mediated induction of embryonic or fetal globin gene expression in definitive erythroid cells (EryD) from the murine fetal liver.

Accelerated differentiation, manifest as changes in cell division, is one proposed mechanism of fetal globin induction. Using a cell-constant fluorescent dye, we observed that SCFAs slow the rate, but do not substantively change the number, of cell divisions as compared with untreated controls. P38 phosphorylation and downstream signaling have also been implicated, as a manifestation of 'stress erythropoiesis', in HbF induction. In our model, Western blots showed that p38 phosphorylation was not unique to exposure with SCFAs. Yet we, like others, found that embryonic globin gene induction was inhibited by all p38 inhibitors tested, in a dose dependant manner. However, we then studied signaling and differentiation, and found that erythroid differentiation and cell division were also blocked by inhibitors of p38. We conclude that p38, while integral to SCFA-mediated induction of embryonic/fetal globin genes, is not specific to this function, and block by p38 inhibitors reflects instead the constitutive role p38 plays in cell division and differentiation. SCFAs may not work primarily through induced stress erythropoiesis, but their actions are dependent upon intact cell proliferation and differentiation.

5. OBSERVATIONAL STUDY OF YOUNG EGYPTIAN ADULTS WITH B-THALASSEMIA MAJOR (B-TM) ON DESFERRIOXAMINE (DFO) TO ASSESS CARDIAC EVENTS AND CARDIAC T2*

MS Elalfy¹, I Abdin¹, A Samir², and D.Salem¹, ¹Ped Hemat/Onc and MRI Unit ²Ain Shams University, ³Radiology Unit Cairo University, Egypt

Background: Cardiac events and death are common in adults with B-TM on desferrioxamine (DF) monotherapy either because of non compliance or ineffectiveness. Aim of study was to record DFO compliance, cardiac events in patients on DFO monotherapy and to evaluate cardiac T2* while on DFO. Methods; all B-TM patients ($n= 322$) aged 6-12 years on 1998 were followed prospectively. All cardiac events whether fatal or not were recorded and on 2008 all patients still on DFO monotherapy not showing symptoms or signs suggestive of cardiac problems and their Echocardiogram was normal ($n=76$) were evaluated for serum ferritin, liver R2*(LIC) and for cardiac siderosis by T2*. Results; on 2008; only 82 were on DFO monotherapy, 112 on combined DFO and DFP, 66 on DFP, 44 on DFX, 12 lost follow-up and 16 died (10 for cardiac problem). During DFO monotherapy for 1520 patient-year only 40% were compliant on DFO; 8/10 cardiac deaths were in DFO group and 12/16 of non fatal events. Out of the Seventy-six on DFO monotherapy (median age of 19 years , 60% were males), SF ranged from 624-8759 (median of 3747ng/ml) , LIC ranged from 4.4-52 mg/g and cardiac T2* was ; <20 ms in 22(28%); 10-20 ms ($n=14$) and <10 ms ($n=8$) ; 37.5% of those <10 ms were compliant on DFO .Conclusion; only 40% of DFO monotherapy were compliant on it , most cardiac events whether fatal or not were on DFO monotherapy ,even compliance on DFO can not prevent severe cardiac siderosis.

6. THE TOLERABILITY, SAFETY AND EFFICACY OF AN ORAL SOLUTION OF DEFERIPRONE IN CHILDREN WITH TRANSFUSIONAL IRON OVERLOAD

EI-Alfy Mohsen, Chan LL, Sari TT, Ah Moy Tan, Lai Angeline, Ng Ivy, Tricta F, El-Beshlawy A, Children Hospital, Ain Shams University, Cairo, Egypt; University of Malaya, Kuala Lumpur, Malaysia; Cipto Mangokusumo National Hospital, Jakarta, Indonesia; Department of Haematology/Oncology, KK Women's and Children's Hospital, Singapore; Department of Paediatric Medicine, KK Women's and Children's Hospital, Singapore; Department of Genetics Service, KK Women's and Children's Hospital, Singapore, ApoPharma Inc. Toronto Canada; Pediatric Hematology Hospital, Cairo University, Cairo, Egypt

Prior to a 6 month study of deferiprone oral solution (Ferriprox) in 100 transfusion-dependent pediatric subjects ≤ 10 years old (Study LA30-0307, sponsored by ApoPharma), limited data existed on the safety and efficacy of deferiprone in young children. To obtain additional data, subjects were transferred into a compassionate use program (LA28-CMP).

To date, Ferriprox oral solution was prescribed at 75-100 mg/kg/day in 108 children (1 - 16 years). The mean duration of therapy was 392 ± 173 days (median = 475; range = 1-689 days). Most subjects were originally switched from deferoxamine, but 28 children were naïve to chelation therapy.

The safety profile was similar to that observed in older subjects. Seven subjects experienced neutropenia [absolute neutrophil count (ANC) $1.5 \times 10^9/L - 0.5 \times 10^9/L$], all resolved despite continuous deferiprone use. Four subjects experienced agranulocytosis [ANC $< 0.5 \times 10^9$] which lasted 1-2 weeks. All subjects recovered either spontaneously or after administration of G-CSF and were discontinued from the program.

The Last Observation Carried Forward (LOCF) method was applied to the serum ferritin data. Over time, there was a significant decrease in serum ferritin value from baseline. At month 15 the mean serum ferritin concentration ($\mu\text{g/L}$) had decreased significantly from 2553 ± 1483 at baseline to 2294 ± 1328 ($p=0.03$).

These results indicate that Ferriprox Oral Solution is well tolerated, safe, and effective in lowering serum ferritin in young children, even when administered for up to 15 months.

7. PRECLINICAL ASSESSMENT OF GENE THERAPY TO BETA-THALASSEMIA SHOWS EFFICACY AND SAFETY OF LENTIVIRAL-MEDIATED GENE TRANSFER IN BM-CD34+ CELLS

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(1) H.San Raffaele-Telethon Institute for Gene Therapy (HSR-TIGET), Milan, Italy; (2) Pediatric Clinic Research Unit, (PCRU-TIGET) H.S. Raffaele, Milan, Italy; (3) Department of Biomedical Sciences, University of Modena and Reggio Emilia, Modena, Italy; (4) Mediterranean Institute of Hematology, Policlinico di Tor Vergata, Rome, Italy

Thalassemias are globally the most common monogenic disorders, affecting thousands of newborn annually. So far, allogeneic bone marrow (BM) transplantation from HLA-matched donors represents the only definitive cure for patients affected by beta-thalassemia major, although limited to patients with compatible donors. For all patients lacking a donor, autologous transplantation of genetically corrected hematopoietic stem cells offers the promise of a definitive cure. Therefore, a relevant issue is to demonstrate feasibility, safety and therapeutic efficacy of gene transfer in preclinical models and patients' cells.

We recently developed a β -globin-expressing lentiviral vector (GLOBE) able to sustain long-term correction of thalassemia in the murine preclinical model (Miccio et al. PNAS, 2008). To move forward with clinical translation, the therapeutic efficacy of GLOBE was tested in the context of human cells. The availability of samples from a large, genetically heterogenous cohort of thalassemia major patients allowed us to perform analyses with statistically significant numbers. No difference in the yield and clonogenic potential of CD34+ cells was found between normal and thalassemic samples ($n=29$). We transduced BM-derived CD34+ cells

(n=19), with a transduction procedure based on short-time cytokine stimulation, and obtained high efficiency of gene transfer (37-98%, mean: 67%), leading to normal levels of HbA in erythroid cells. Following pre-activation by cytokines, we observed changes in the proportion of committed progenitors indicating the relevance of analyzing the impact of the transduction on the cells to be infused in future clinical trials. For a global analysis, we generated data from microarrays for BM-derived CD34+ cells of 47 samples, including untreated, pre-activated, thalassemic and normal controls. In order to assess the risk of integration in potentially dangerous genes we sequenced and mapped the GLOBE integration sites in the human genome, from DNA of thalassemic CD34+ cells. Analysis of integration events showed preference for intragenic regions, without hot spots in protooncogenes, tumor-suppressor or cell cycle related genes. Cross-annotating the expression category for flanking host genes revealed that GLOBE preferentially lays into proximity of expressed genes. Finally, the genotoxic potential of LCR-containing globin vectors in perturbing the expression of genes flanking the integration sites was tested in human erythroid cells. Overall, these results provide evidence of efficacy and safety for the use of GLOBE in clinical gene therapy of thalassemia.

8. ALTERNATING DEFERASIROX-DEFERIPRONE THERAPY: A CHANCE FOR “HARD-TO-CHELATE” THALASSEMIA MAJOR PATIENTS

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At present three drugs are available to reduce iron overload in Thalassemia Major patients: Deferoxamine (DFO), Deferasirox (DFX) and Deferiprone (DFP). Each of these drugs can be used as monotherapy: DFO and DFP are also used together, either given on the same day or on different days. However, some patients are unable to tolerate monotherapy – and consequently dual therapy – with any of these drugs. We report the successful results of a daily alternating DFX-DFP therapy administered in two Thalassemia Major patients over a period of **one year**. These patients resulted untreatable with monotherapy and with dual DFO–DFP therapy due to systemic reactions and fever for DFO, respectively proteinuria and skin reactions for DFX and neutropenia and arthralgia for DFP. These symptoms reappeared after several re-challenges and were not avoidable. The only option was to administer intravenous DFO, but the impact on the patient's quality of life would be heavy.

Patient 1 is a 20-year-old female presented ferritin level of 6150 ng/ml with a MRI-LIC of 8.4 mg Fe/g dw and cardiac T2* 24 mms. After one year of the alternating therapy at dosage of DFX 30 mg/Kg/daily and DFP 90mg/Kg/ into 3 doses/day her ferritin value decreased to 2300 ng/ml, MRI-LIC to 3.8 mg Fe/g dw and cardiac T2* remained stable.

Patient 2 is a 33-year-old female presented a ferritin level of 600 ng/ml, MRI-LIC of 1.82. mg Fe/g dw, cardiac T2* 28 mms. We prescribed daily alternating DFX 25 mg/kg/day and DFP 75 mg/kg/ into 3 doses/day. Over a period of one year her ferritin value, LIC and cardiac T2* remained stable. The patients' clinical and biochemical status was closely monitored. The regimen was well tolerated, neither kidney function alteration, nor arthralgia or any other safety problems were noted.

To our best knowledge this is the first report describing alternating therapy DFX-DFP. We think this approach should be further investigated since it could be a new chance to approach lifesaving chelation therapy for those patients who hardly tolerate both monotherapies and dual deferoxamine-deferiprone therapy.

9. THE CANDIDATE SNPS BIOMARKER FOR DISEASE SEVERITY IN α -THALASSEMIA

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Compound heterozygous α -thalassemia/HbE is the most common α -thalassemia disease in Southeast Asia and worldwide. The patients have a wide variation of clinical presentation ranging from nearly asymptomatic to transfusion-dependent thalassemia major. However, excluding the known major modifier factors which involved in the degree of imbalanced globin chains, phenotypic variability in α -thalassemia/HbE patients remains unpredictable. Genome-wide association study of approximately 110,000 gene-based single nucleotide polymorphisms (SNPs) were explored in 487 α -thalassemia/HbE patients who have mild and severe clinical presentations. Forty-five SNPs in the α -globin gene cluster were found to have a strong association with the disease severity. Among these, 2 SNPs in the LCR and 1 SNP in the 3'UTR of α -globin gene, rs3888708 (A/C), rs11036641 (A/G), rs35043458 (C/A) had the highest association (p-value <10⁻⁴). The three SNPs were genotyped using reverse dot-blot hybridization technique in another cohort of 71 mild and 93 severe cases of α -thalassemia/HbE. The result showed correlation of the 3 SNPs with disease severity with the odd

ratio 1.90 (95% CI; 0.73 – 4.90) in genotype AA, AA, CC and 3.25 (95% CI; 0.66 – 15.79) in genotype AC, AA, CC. Taken together, the 3 SNPs may be applied as a candidate SNPs biomarker for severity prediction in α -thalassemia disease.

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10. ROLE OF MACROPHAGES ON THE APOPTOSIS OF α -THALASSEMIA/Hb E ERYTHROID PRECURSOR CELLS

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Ineffective erythropoiesis in thalassemias is in part due to accelerated apoptosis of the erythroid precursors. Erythroid apoptosis in α -thalassemia is approximately 15- fold above the healthy controls. Erythrokinetic studies performed in β -thalassemic patients have shown that the bone marrow is the main site of erythroid cells death. The increased number of macrophages, which are in the enhanced state of activation in thalassemic bone marrow, has suggested an important role of macrophages in thalassemic erythroid cells apoptosis. In this study the effect of macrophages to erythroid apoptosis and enhanced phagocytosis was determined using co-cultures of erythroid cells (E) with native or LPS activated macrophages (M) at E: M ratio 3:1. The result showed the increased phagocytosis, both in normal and α -thalassemia/HbE erythroid cells with the increasing time of co-cultures, and the increase was greater in α -thalassemia/HbE. In addition annexin V assay demonstrated the significantly increased phosphatidylserine (PS) exposure of thalassemic and normal erythroid precursor cells when these cells were co-cultured with activated macrophages, and the effect was more pronounced in α -thalassemia/Hb E. The native stage of α -thalassemia/Hb E macrophages also induced more PS exposed erythroid cells in comparison to normal macrophages. This effect, shown by percent apoptotic changes, still existed when thalassemic macrophages were cross co-cultured with normal and α -thalassemia/HbE erythroid precursor cells. The result suggested that thalassemic macrophages had a greater effect to PS exposure of erythroid precursor cells than normal macrophages.

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11. BP1 BINDING SITE POLYMORPHISMS AS A POTENTIAL GENETIC MODIFIER OF BETA-THALASSEMIA DISEASE SEVERITY

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The binding of the repressor protein BP1 (beta protein 1) to its binding site on the beta-globin promoter involves in the negative regulation of the adult beta-globin gene expression. Several line of evidence has shown that variations in the BP1 binding site motif, a tandem (AC)n(AT)xTy repeats, affect the binding affinity of BP1 protein and associate with the severity of sickle cell anemia. In beta-thalassemia, the degree of imbalanced globin chain synthesis correlates with the disease severity. To evaluate the contribution of BP1 binding site polymorphisms in modifying the beta-thalassemia disease severity, DNA sequencing for the beta-globin gene promoter containing (AC)n(AT)xTy motif region was performed in a well-characterized cohort of 400 Thai/Chinese beta-thalassaemia/HbE patients and 50 homozygous hemoglobin E. Ten different configurations of the BP1 binding site variants were found in the cohort. Four motifs; (AC)2(AT)9T5, (AC)2(AT)7T7, (AC)2(AT)8T5 and (AC)3(AT)7T5, accounted for 88.3% of all identified variants with frequencies of 42.4%, 20.0%, 14.6% and 11.3%, respectively. The (AC)2(AT)9(T)5 allele is in strong linkage disequilibrium with the Hb E allele. Comparison of the BP1 binding site variants frequencies in 200 severe versus 200 mild patients revealed no significant difference in the distribution. In addition, there was no association between either variants and clinical phenotypes or fetal hemoglobin level. This study indicates that the variation of BP1 binding site motif on the beta-globin gene promoter is not associated with the beta-thalassemia disease severity in Thai/Chinese population.

12. EXOGENOUS TRANSFERRIN AMELIORATES DISEASE IN $\alpha\alpha$ -THALASSEMIC MICE

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-thalassemia patients develop iron overload caused by uptake of labile plasma iron by parenchymal cells in a dysregulated manner which results in major morbidity and mortality. Because normal erythropoiesis requires transferrin-bound iron uptake via the transferrin receptor, we hypothesized that anemia in -thalassemia results, in part, from insufficient circulating transferrin, which leads to expanded, but ineffective, erythropoiesis. We determined the capacity of exogenous transferrin to modulate iron metabolism and erythropoiesis in mice with -thalassemia. Providing exogenous transferrin to increase the efficiency of iron transport has several potential advantages: 1) Transferrin is able to sequester unbound iron, 2) iron is maintained in a redox-inactive form, and 3) iron is prevented from precipitating in tissues. Using human transferrin administered by daily injections in -thalassemic mice, we compared parameters in untreated and age and gender matched transferrin-treated mice. Exogenous transferrin significantly improved pathological characteristics of -thalassemia. Specifically, transferrin 1) normalized red cell survival, 2) increased hemoglobin production with concomitantly decreased reticulocytosis, erythropoietin levels, and extramedullary erythropoiesis in the liver, 3) normalized levels of labile plasma iron, and 4) increased hepcidin expression. Furthermore, transferrin improved the ability of erythroid precursors to mature, indicating its role in increasing the effectiveness of erythropoiesis in -thalassemia, and lead to a reversal of splenomegaly in transferrin-treated mice. Lastly, hepcidin expression increased despite low transferrin saturation in mice with decreased extramedullary erythropoiesis; providing further evidence for the existence of an “erythroid regulator” of hepcidin. Together, these results indicate that transferrin is a limiting factor contributing to anemia in these mice. We speculate that transferrin may have several potential uses not previously considered, e.g. for treatment of patients with diseases of concurrent anemia and iron overload in which additional transferrin could be used to abrogate ineffective erythropoiesis by redirecting iron from parenchymal deposition to erythropoietic machinery for hemoglobin synthesis. A novel approach would greatly benefit this patient population for whom standard management has consisted of transfusion followed by chelation therapy for the last half-century.

13. REVERSAL OF HYPOGONADISM AND GLUCOSE METABOLISM DISTURBANCES IN β -THALASSEMIA MAJOR PATIENTS AFTER COMBINED CHELATION AND DECREASE OF IRON LOAD TO NORMAL LEVELS

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The frequency of endocrinopathies in β -thalassemia major patients (TMps) increased as survival improved. Hypogonadism is the most common (40-91%) and dramatically affects patient quality of life because of fertility problems and the impact on their psychological outcome. On the other hand, diabetes has a frequency of 6-28% and an established effect on cardiovascular disease. Data regarding the relationship of endocrine dysfunction and chelation therapy are conflicting. The primary endpoint of the present study was to place all TMps in a negative iron balance while observing the evolution of endocrine complications secondary to iron overload.

50TMp, mean age 30.8 ± 2.03 , were previously maintained on subcutaneous desferrioxamine (DFO) monotherapy and switched to an intensive combined chelation with DFO: 40-60mg/kg/d and Deferiprone (DFP: 75-100mg/kg/d), on an individually tailored regimen. They were investigated initially and annually thereafter. The current analysis reports the evaluation after 5-8 years. Iron overload was estimated by mean ferritin and hepatic iron quantification by MRI T_2^* L and Liver Iron Concentration (LIC) calculated by Ferriscan. Endocrine function was assessed by dynamic tests (GnRH, OGTT) and hormonal screening. Statistical analyses were performed using SPSS ($p < 0.05$ was considered significant).

1. *Hypogonadism*: Initially, 14/24 males (58%) were hypogonadic on testosterone therapy. After intensive combined chelation, hypogonadic TMps had normal mean testosterone (4.6 ± 0.7 vs. initial 1.5 ± 0.5 ng/ml, $p < 0.0001$); this was correlated with normalization of mean ferritin levels ($3,349 \pm 882$ vs. 108 ± 32 g/L, $p = 0.004$), mean MRI T_2^* L (32 ± 2 vs. initial 6.5 ± 2 msec $p < 0.0001$) & LIC (0.9 ± 0.05 vs. initial 19.5 ± 7 mg/g dry weight, $p = 0.003$) ($r = -0.634$ $p < 0.04$, $r = 0.668$ $p < 0.03$, $r = -0.679$ $p < 0.03$ respectively). 7 of these 14 (50%), also normalized their LH-FSH response during GnRH test and discontinued testosterone injections. One became the father of twins. In the eugonadal group, no new cases of hypogonadism were observed and similar correlations were observed with the increase in mean testosterone levels (6.8 ± 0.6 vs. initial 3.6 ± 0.5 ng/ml, $p < 0.0001$). With DFO monotherapy 19/26 females TMps (73%) were hypogonadic on hormone replacement therapy. After combined chelation, a significant increase of FSH excretion (AUC FSH 373 ± 37 vs. 733 ± 58 , $p < 0.0001$) was observed; this was strongly correlated with normalization of MRI T_2^* L (35 ± 1 vs. 10 ± 4 msec, $p < 0.0001$) and mean Ferritin (114 ± 12 vs. $2,696 \pm 587$ g/L, $p < 0.0001$) ($r = 0.723$ $p < 0.04$, $r = -0.936$ $p < 0.01$ respectively). A correlation was also observed between the increase in LH (1.9 ± 0.3 to 3.8 ± 0.5 mIU/mL $p < 0.000$) and decrease of LIC (11 ± 4 to 0.9 ± 0.05 mg/g dry weight, $p = 0.002$) ($r = 0.885$ $p < 0.04$). 6 females (32%) became pregnant, 2 spontaneously and 4 by IVF.

2. *Glucose metabolism disturbances and Diabetes*: Among the 50 TMps in DFO monotherapy 6 had Insulin-dependent Diabetes, 14 had Diabetes-type 2 (Glucose 0'>126mg/dl, 2h>200mg/dl), 19 had Impaired Glucose Tolerance (IGT: Glucose 2h>140<200mg/dl). Following combined chelation 9/14 Diabetic-2 (64%) and 17/19 IGT (90%) normalized their glucose metabolism. In IGT TMps there was a significant decrease in Glucose secretion (AUC Glucose 13,399±481 vs. 18,101±777, p<0.0001) and an increase in Insulin secretion (AUC Insulin 6,320±665 vs. 4,478±453, p<0.05) which correlated strongly with the decrease in ferritin [2,690±393 to 113±19g/L, p<0.0001 (r= -0.592 p<0.02, r=0.568 p<0.02 respectively)]. There was also a strong correlation between the decrease in glucose 2h from 152±7 to 105±6mg/dl p<0.0001 and the normalization of MRI T₂*L (35±2 vs. 8±2 msec, p<0.0001) and LIC (0.9±0.05 vs. 13±4mg/g dry weight, p<0.008) (r=0.629, p<0.01 & r= -0.619, p<0.01 respectively). Conclusion: Long-term intensive combined chelation with DFO & DFP cleared iron overload. This study provides insight in the relationship between iron load and the reversal of Hypogonadism and Glucose metabolism abnormalities. This analysis highlights the possible role of Deferiprone which was added to DFO monotherapy and reversed the course of these endocrinopathies. Further studies are needed.

14. FRUCTOSAMINE IN THE MANAGEMENT OF ABNORMAL GLUCOSE METABOLISM IN β-THALASSAEMIA MAJOR PATIENTS

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Abnormal glucose metabolism is a frequent complication in Thalassemia major patients (TMp). The main underlying mechanisms are either insulin deficiency due to the direct toxic damage of iron on pancreatic beta cells, and/or long-standing insulin resistance due to iron overload on different tissues. Usually patients are asymptomatic but it may manifest from mild glucose intolerance to overt diabetes which has an established effect on cardiovascular disease. The widely accepted glyco-hemoglobin test (A1C test) is not accurate in patients with condition that affects the average age of red blood cells (RBCs), such as hemolytic anemia or blood loss. In these cases, fructosamine test, can be used to monitor glucose control according to American Diabetes Association (ADA). This test measures glycated serum protein which is proportional to blood glucose levels and remains unchanged for 14 to 21 days providing an overall view of glucose levels for the past few weeks.

100 TMp, 49 males 51 females, mean age 31.8±2.03 undergo a fructosamine test for their glycemic control. They were classified according to the ADA criteria after an oral glucose tolerance test (OGTT). Although serum fructosamine values reflect mean blood glucose levels over a couple of weeks, evaluated in the context of the patient's total clinical findings, may add up useful information in TMp follow-up. In all Insulin-dependent Diabetic TMp, fructosamine levels were the highest. Abnormal levels were also observed in Diabetic TMp treated with Sulfonylureas. Unlikely to Diabetic TMp treated with Biguanides & Incretin either as monotherapy or combined therapy, fructosamine levels were normal. Among normal patients mean fructosamine levels were lowest. The results of our study are shown in table 1:

N=100	N	Mean Fructosamine Normal:122-236µmol/l
Insulin-dependent Diabetes	5	363,8 ± 65
Diabetes type II (Glucose 0'>126mg/dL & 120'>200mg/dL)	17:	
- treated with Sulfonylureas	3	311 ± 47,8
- treated with Biguanides & Incretin	11	195,4 ± 22,2
- with Incretin therapies	3	174,3 ± 20,8
Impaired Glucose Tolerance (IGT) (Glucose 0'>100<126mg/dL & 120'>140 & <200mg/dL	11	175,3 ± 11,5
Normal Glucose Metabolism	67	159,7,1 ± 27,9

Although the information provided by a fructosamine test is useful, the test is not widely administered. Our results lend support to the idea that fructosamine testing in TMp may be used as a target for glycemic control, like A1C testing is recommended by ADA & AACE (American Association of Clinical Endocrinologists) in normal population. Further studies are needed to confirm this speculation. Also trends may be more important than absolute values. In certain circumstances needing shorter-term assessment, such as pregnancy or recent adjustment of a treatment plan, the fructosamine test has advantages. Other benefits are predicting the patient's risk of developing complications such as macrovascular disease, nephropathy, neuropathy and retinopathy. Finally fructosamine test can show TMp the effectiveness of their decisions on diet, exercise or medication and encourage them to continue their performance.

15. ROLE OF KLF4 AND KLF12 IN γ -GLOBIN GENE REGULATION

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Kruppel-like factors (KLFs) are a family of Cys₂His₂ zinc-finger DNA binding proteins that regulate gene expression through CACCC/GC/GT box binding in gene promoters. The CACCC element is critical for the developmental regulation of the human γ -globin and δ -globin genes and studies are being done to ferret out various factors that bind this region and modulate gene activity. We recently identified two Kruppel-like factors, KLF4 and KLF12 whose expression levels decreased based on microarray-based gene profiling, concomitantly with decreased γ -globin expression during erythroid maturation. Decreased expression of both factors was further confirmed using quantitative PCR (qPCR) analysis. KLF4 and KLF12 mRNA levels decreased 56-fold and 16-fold respectively by day 28 compared to levels in day 7 erythroid progenitors. We next determined if KLF4 and KLF12 bind the γ -globin CACC box by electrophoretic mobility shift assay (EMSA) using nuclear proteins extracted from K562 cells and a [³²P] labeled γ -CACC probe located between -155 to -132 relative to the γ -globin gene cap site. Three DNA-protein complexes were observed. The specificity of these interactions was confirmed by competition reactions in which preincubation with excess unlabelled γ -CACC oligonucleotide effectively abolished the formation of all DNA/protein complexes; addition of nonspecific oligonucleotide had no effect on binding activity. Addition of polyclonal KLF4 or KLF12 antibodies to the EMSA reaction resulted in a marked decrease in intensity of all DNA-protein complexes suggesting both KLF4 and KLF12 are present. Additional studies were performed to determine the effect of the known fetal hemoglobin inducer hemin on KLF gene expression in K562 cells. Hemin stimulated γ -globin transcription while increasing KLF4 and KLF12, 66-fold and 4-fold respectively (p<0.05). Hemin treatment in KU812 erythroleukemia cells which actively transcribe both δ - and γ -globin, also produced a 10-fold increase (p<0.05) in KLF4; KLF12 levels were not changed. Our preliminary data suggest these KLFs might play a role in γ -globin regulation. siRNA mediated gene silencing studies are underway to determine if KLF4 and/or KLF12 play a direct role in γ -globin gene regulation. This mechanism could provide important molecular targets for fetal hemoglobin reactivation. This will be highly significant towards developing therapeutic strategies for hemoglobinopathies like sickle cell anemia and δ -thalassemia.

16. CARDIOVASCULAR COMPLICATIONS IN β -THALASSEMIA INTERMEDIA: ROLE OF CHOLESTEROL AND IRON METABOLISMS

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Cardiovascular complications represent the primary cause of mortality and one of the main causes of morbidity both in thalassemia major (TM) and in thalassemia intermedia (TI). In TM, iron overload is considered the main cause of these complications. Cardiovascular involvement in TI, however, is quite different. Patients live longer and are usually transfusion-independent, at least for the first decades of their life. Several factors have been reported to interfere in the pathophysiology of cardiovascular abnormalities in TI patients. However, at the present, no factor emerges as major responsible for atherosclerotic risk in these patients. In the present study, total and HDL-cholesterol (TC and HDL-C) and hepcidin levels in serum and cholesterol ester (CE) content in peripheral blood mononuclear cells (PBMCs) were evaluated in 18 β -TI patients, aged 18 to 54 years. Eighty age-matched blood donors, free from **thalassemia**, were utilized as controls. Neither patients nor controls were positive for viral RNA-HCV. The mRNA levels of the main genes involved in cholesterol homeostasis and of hepcidin, IL1- α and TNF- α were also evaluated in PBMCs of the above subjects. β -TI had significantly lower total and HDL-C and lower serum hepcidin levels compared with controls. Lower HDL-C levels were associated with higher levels of erythropoietin and ferritinemia. Interestingly, correlation analysis revealed a significant negative correlation between HDL-C levels in serum and intracellular CE in PBMCs. The expression of genes involved in the CE cycle also significantly changed in TI: mRNA levels of acyl-coenzyme A: cholesterol acyltransferase (ACAT1), the enzyme responsible for intracellular esterification, and LDL-receptor were increased, while sterol regulatory element binding protein-2 (SREBP2), neutral cholesterol ester hydrolase (nCEH) and ATP binding cassette-A (ABCA1) were decreased. This molecular pattern is widely found in cardiovascular complications secondary to atherosclerotic lesions. Hepcidin mRNA levels were reduced in TI patients and were even lower than those observed in TM receiving regular blood transfusions. Lower hepcidin mRNA levels were also associated with lower mRNA levels of IL- α ; tumour necrosis factor alpha (TNF- α) did not change in TI.

These results may, at least in part, explain the high incidence of atherogenic cardiovascular complications observed in β -TI. We conclude that the analysis of the expression of genes involved in the CE and iron cycles may represent a sensitive index to evaluate the potential atherogenic risk in β -TI patients.

17. LACK OF BINDING OF DEFERIPRONE TO BOVINE SERUM ALBUMIN

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The iron chelating pharmaceutical deferiprone was tested for its binding to highly purified Bovine Serum Albumin (BSA) by an equilibrium dialysis technique. All solutions were prepared in 4-morpholinepropane sulfonic acid (MOPS), 0.1M, pH 7.4, buffer, and dialysis was carried out for 24 hours at 37°C. Using a range of initial concentrations of deferiprone, with a fix BSA concentration (3.5g/L), the final concentration of deferiprone was determined by quantitative UV spectrophotometry. No significant binding of deferiprone to BSA was observed. Since Human Serum Albumin (HSA) has close homology to BSA, it is likely that deferiprone does not significantly bind to HSA. Our results can help explain the observed pharmacokinetics of deferiprone.

18. RESPONSE TO DEFERASIROX- AN INDIAN EXPERIENCE

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Deferasirox (DFX) is a tridentate iron chelator with good oral bioavailability, requiring single daily dosing. From June 2008, we subjected 50 transfusion dependent thalassemic patients requiring iron chelation, to DFX at dose of 20 mg/kg/day. Serum ferritin, CBC, SGPT, Creatinine, urine, were recorded, and all patients had Liver and Cardiac iron quantification done by MRI T2* on Siemens 1.5 Tesla machine, before starting therapy. Mutation and XMN polymorphism analysis were done in all. The mean pre DFX therapy ferritin level was 3528.6±1958.6 ng/dl and mean hepatic T2* iron value was 2.7 ms (normal > 6.3 ms). 92% cases demonstrated moderate to severe hepatic iron overload, whereas in 50% there was moderate to severe cardiac siderosis (T2* < 10ms). 9 cases were also receiving hydroxyurea to facilitate fetal Hb production. 27/50 cases (54%) demonstrated transient increase in serum ferritin level (mean = 4919 ng/dl) from baseline on initiation of therapy. As there was no drop in ferritin at dose of 20 mg/kg, DFX was increased to 30mg/kg. 30/50 cases (60%) demonstrated a consistent fall in ferritin on this dose (mean = 2584ng/dl). 4 patients (8%) had transient skin rashes, whereas 1 had significant rise in SGPT level, warranting discontinuation of therapy. Transient increase, but within the normal range of serum creatinine, was noted in one.

This study demonstrates that dose of 20 mg/kg DFX was ineffective whereas doses of ≥ 30 mg/kg are well tolerated and effective in reducing serum ferritin. We recommend further pharmacokinetic and pharmacogenomic studies of DFX in Indian thalassemic population

19. ANCREATIC IRON AND PANCREATIC FUNCTION IN THALASSEMIA

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As patients with thalassemia major survive into middle age and beyond, there is greater need to address endocrine complications such as diabetes mellitus. Pancreatic iron deposition is common in thalassemia major patients and correlates with cardiac iron deposition. Unlike in the heart, endocrine dysfunction may not be completely reversible once functional assays become abnormal. Our aim of this study was to compare pancreatic R2* (the reciprocal of T2*) measurements with the results of an oral glucose tolerance test (OGTT) to determine the relationship between pancreatic iron and pancreatic dysfunction. We hypothesized that pancreatic R2* would be predictive of abnormal pancreas function.

We recruited 39 patients with thalassemia major to participate in a frequently sampled OGTT. The average pancreas R2* was 289.2 ± 257.3 Hz (Normal < 27 Hz), cardiac R2* was 92.8 ± 80.1 Hz (Normal <50 Hz), and hepatic iron concentration (HIC) was 15.4 ± 18.0 mg/g dry weight. Pancreatic R2* correlated with cardiac R2* ($r^2 = 0.55$, $P = <0.0001$) in this population. Of the 39 patients enrolled, 10 had diabetes, 9 had impaired glucose tolerance, and the remaining 20 had normal glucose tolerance. Disposition index, a surrogate for islet cell function, was negatively correlated with pancreas R2* ($r^2 = 0.15$, $P = <0.0163$). All of the patients with diabetes had a pancreatic R2* >150 Hz, as did 6 of the patients with impaired glucose tolerance, and 8 normal patients. For pancreas R2*, the area under the ROC curve was 0.70. These data confirm that pancreatic R2* appears to identify patients having a higher prevalence of diabetes, however, many

patients with increased pancreatic iron have normal pancreatic function. Serial studies are needed to determine whether elevated pancreatic iron predicts future decline in pancreatic function.

20. DEFERASIROX IN NORTH INDIAN β -THALASSEMIA MAJOR: A PRELIMINARY REPORT

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Deferasirox is a relatively new iron chelator approved by USA FDA in children >2years age. Prospective studies in Asian Indian children have not been reported. The aim was to analyse the short term side effects of Deferasirox in north Indian β -thalassemia major patients. The β -thalassemia patients receiving regular transfusions in thalassemia ward of APC were included in the study. Side effects monitoring was done with monthly transaminases and serum creatinine levels. Hemoglobin levels were done prior to blood transfusion. 30 patients of transfusion dependent thalassemia were eligible for final analysis. The M:F ratio was 23:7; and the age ranged from 2.0 to 21 years & serum ferritin level at start of therapy was 2657.7 ± 1414.6 (mean \pm SD). Mean dose of deferferasirox was 21.57 mg/ kg/ day (range from 17.2 to 27.2 mg/kg/day), common side effects noted were gastrointestinal in 5(16.6%) and skin rash in 2(6.6%) over 12 months period. There was rise in serum creatinine in two patients and treatment was interrupted in one. No significant cytopenias were observed. In 13/30 patients, initial rise in serum ferritin was observed. Thus, deferferasirox is a relatively safe oral iron chelator in Asian-Indians with minor short term side effects. Initial rise in serum ferritin can occur. Treatment requires individualization with careful dose escalation and proper monitoring for the side effects.

21. A POST MARKETING OBSERVATIONAL STUDY OF CHILDREN (6 YEARS OR OLDER) TREATED WITH EXJADE (DEFERASIROX ICL 670)-PRELIMINARY DATA

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Exjade is a once daily oral iron chelator that was developed for the treatment of transfusional iron overload and became licensed in Canada in 2006 for use in children over the age of 6 years. The purpose of this study is to evaluate the long-term response of deferferasirox in children aged 6 years or older treated with Exjade for chronic iron overload related to transfusions used in the treatment of transfusion dependent anemia.

To date, 27 subjects with transfusional hemosiderosis treated with Exjade were enrolled. Patient-reported outcome measures questionnaires were given after the written consent/assent was obtained. Demographic information such as age, height, weight, previous chelation therapy and medical diagnosis were collected. Laboratory evaluations such as serum creatinine, ALT, serum ferritin, liver iron assessments were also collected. Statistical analysis was done by descriptive statistics and paired t-test.

Of the 27 subjects, 18 were female and 9 were male and median age for the patients was 14 ± 5.3 . All of the subjects were on desferal chelation therapy before the start of the Exjade, Patient-reported outcome measures analysis showed that out of 27, 14 subjects were very satisfied, 8 satisfied and only 1 subject was dissatisfied with the Exjade. 19 subjects reported it to be very convenient. The main reason for the Exjade treatment preference was more convenient in administration. Other reasons given were relief of not having pain associated with injections and improved sleep patterns.

Further data will be collected after 12 and 24 months to evaluate their long-term responses.

22. REDUCTION AND PREVENTION OF MYOCARDIAL SIDEROSIS WITH DEFERASIROX (EXJADE®) IN REGULARLY TRANSFUSED PATIENTS WITH β -THALASSEMIA

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In a 1-year, prospective, multicenter study, the efficacy of deferasirox (Exjade®) for reducing (n=114) or preventing (n=78) myocardial siderosis was assessed in β -thalassemia patients aged ≥ 10 years undergoing regular blood transfusions. Deferasirox was initiated at 30 mg/kg/day (treatment arm) or 20–30 mg/kg/day (prevention arm), with dose adjustments of 5 or 10 mg/kg/day based on serum ferritin (SF) trends, month-6 myocardial T2*, and safety parameters. In the treatment arm (mean dose 32.6 \pm 4.0 mg/kg/day), geometric mean \pm CV myocardial T2* improved significantly from baseline to 1 year (11.2 ms \pm 40.5% vs 12.9 ms \pm 49.5%, P<0.0001; +16%). LVEF was unchanged but both mean liver iron concentration (LIC) and absolute change in median SF were reduced by -6.6 ± 9.9 mg Fe/g dry weight (dw) and -1257 ng/mL, respectively (both P<0.0001). In the prevention arm (mean dose 27.6 \pm 6.0 mg/kg/day), geometric mean \pm CV myocardial T2* was unchanged from baseline to 1 year (32.0 ms \pm 25.6% vs 32.5 ms \pm 25.1%, P=0.56, +2.0%), and mean LVEF increased (+1.9; P<0.0001). Both mean LIC and absolute change in median SF were reduced by -7.2 ± 10.5 mg Fe/g dw and -1048 ng/mL, respectively (both P<0.0001). Most drug-related adverse events (AEs) were mild to moderate. There were two serious AEs in the treatment arm (one nephritis, one renal tubular disorder); both resolved after drug discontinuation. No patients died in either arm. Eight patients had non-progressive increases in serum creatinine (n=6), or alanine aminotransferase (n=2); all but one (creatinine) were in the treatment arm. In conclusion, 1 year of deferasirox was effective for the prevention and reduction of mild, moderate or severe myocardial siderosis in heavily transfused patients with β -thalassemia, and was generally well tolerated.

23. DEFERASIROX IS A SAFE AND EFFECTIVE IRON CHELATOR IN THALASSEMIA PATIENTS POST BONE MARROW TRANSPLANTATION

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Iron overload has an adverse impact on survival in children undergoing hematopoietic stem cell transplantation (HSCT). We report the use of the oral iron chelator deferasirox as an effective chelating agent in the post HSCT period in two pediatric patients with transfusion dependent thalassemia. Both patients had been on a chelation protocol for at least 1 year prior to HSCT; chelation was stopped prior to HSCT. The patients were monitored during the HSCT for complications including sepsis, veno-occlusive disease, and graft versus host disease. Both children engrafted completely and were transfusion independent with 100% donor chimerism at 100 days post transplant. Serum ferritin levels were 2574 ng/ml and 2215 ng/ml respectively at 6 months post HSCT. Both patients were started on the oral iron chelator deferasirox at 20 mg/kg/day. LIC measured by MRI at approximately 1-year post HSCT were 18 mg/g dry wt of liver & 14.3 mg/g dry wt of liver respectively. Monthly laboratory tests included blood counts, blood urea and creatinine, liver function testing and urinalysis. Iron chelation was discontinued approximately 1 year after initiation of deferasirox, post HSCT. Both patients tolerated the medication well and had an excellent response with a decrease in ferritin levels (510 ng/ml and 533 ng/ml respectively). Post treatment LIC in the first patient was 4.4 mg/g dry wt of liver; the results of LIC in the second patient are pending. There was no observed toxicities (renal or hepatic) secondary to deferasirox in either patient.

Elevated liver iron levels post HSCT can lead to hepatic toxicity including long term changes such as cirrhosis. This is the first report of the use of deferasirox as an effective and safe iron chelator in the post HSCT period for pediatric patients with thalassemia major. Larger studies are required in the pediatric population to further assess the role of this chelator, versus desferoxamine or phlebotomy, to decrease iron burden in the post HSCT setting.

24. MACROPHAGE DEPLETION LEADS TO IMPROVEMENT OF THALASSEMIC PHENOTYPE

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Macrophages represent an important link between erythropoiesis and iron metabolism. However little is known about the role of macrophages under conditions of ineffective erythropoiesis such as in thalassemia intermedia (TI). We aimed to study the role of macrophages on erythropoiesis in mice affected by TI using clodronate liposomes, which eliminates macrophages in spleen, liver and bone marrow. Clodronate treatment induced high degree of mortality in TI mice (60-70%), while no death was observed in wt mice. Death was associated with thrombosis. TI patients might be affected by hypercoagulable state with high risk of thrombosis. We hypothesize that treatment with clodronate enhances this process. To test this hypothesis, TI animals were co-treated with clodronate and antioxidant or anticoagulant agents. Both these treatments were able to partially prevent mortality (31% vs 65% survival). Interestingly, survivors of clodronate treatment exhibited improvement of the thalassemic phenotype, characterized by increased Hb (~ 1.5 g/dL), HCT ($\sim 7\%$) and RBC count ($\sim 2 \times 10^6$ cells/ul) in peripheral blood, partially associated with increase RBC half-life. Ineffective erythropoiesis and splenomegaly were also ameliorated ($\sim 40\%$ decrease in spleen size). Our data suggests that macrophages may act as negative modulators of erythropoietic development in thalassemia. One hypothesis is that macrophages sense erythroid

maturation/differentiation and act as modulators of this process. A second hypothesis is that macrophages, under conditions of iron overload, produce inflammatory cytokines that further impairs erythropoiesis in thalassemia.

25. SAFETY OF THE ORAL IRON CHELATOR DEFERIPRONE IN THALASSEMIA MAJOR PATIENTS WITH MILD IRON OVERLOAD

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Iron chelation toxicity may be in part due to chelation of iron which is required for various biological processes. This pharmacological toxicity is considered the main reason for the greater toxicity observed during chelation therapy in patients with mild than in patients with moderate or severe iron overload. Deferiprone has relatively lower affinity for iron than deferoxamine and deferasirox. In conditions of mild iron load, deferiprone has the ability to donate iron to physiological acceptors. Based on its characteristics, contrary to what is observed with deferoxamine, the pharmacological toxicity of deferiprone may not be higher in patients with mild body iron load. In this database review, we assessed the occurrence of drug-related adverse events (ADRs) during treatment with deferiprone in patients with mild iron overload.

We assessed the number of ADRs in thalassemia major subjects who were treated with deferiprone for at least 6 months and had at least two assessments of serum ferritin (SF) levels below 1,000 µg/L or 500 µg/L during the treatment period. The median SF value during the period between first and last assessment below the threshold levels was calculated for each subject. Those subjects with a median value less than 1,000 µg/L or 500 µg/L were categorized as Cohort 1 (N=59) or Cohort 2 (N=25), respectively. Incidence of ADRs in the two cohorts during the period was compared to the incidence in the subject population with SF greater than the threshold levels (N=342 and 376, for Cohort 1 and Cohort 2, respectively).

Overall, there was a significantly lower incidence of ADRs in the two cohorts than in the subject population with higher SF levels (63.6 vs. 160.4 per 100 person-years for Cohort 1 and 29.3 vs. 149.9 per 100 person-years for Cohort 2; $p < 0.05$). Relatively low SF levels were not associated with increased incidence of ADRs including increased ALT levels (1.7% vs. 8.2% for Cohort 1 and 0 vs. 7.7% for Cohort 2), GI intolerance (10.2% vs. 30.4% for Cohort 1 and 4.0% vs. 30.3% for Cohort 2), increased appetite (1.7% vs. 5.6% for Cohort 1 and 0 vs. 5.3% for Cohort 2), musculoskeletal and connective tissue disorders (15.3% vs. 16.4% for Cohort 1 and 12.0% vs. 16.0% for Cohort 2) and increased weight (5.1% vs. 2.6% for Cohort 1 and 4.0% vs. 2.9% for Cohort 2). Those differences were not statistically significant except for GI intolerance ($p < 0.05$). No neutropenia or agranulocytosis episode was reported in the two cohorts.

These results confirm that the toxicity of deferiprone is not higher in patients with mild body iron load.

26. CHARACTERIZATION AND POSSIBLE INVOLVEMENT OF THE CALPAIN-CALPASTATIN SYSTEM AS DISEASE MODIFIERS OF β -THALASSEMIA/HB E DISEASE

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Calpain-mediated proteolysis of unmatched α -globin chains is suspected to reduce their accumulation and the precipitation in β -thalassemia/HbE erythroid precursor cells, and thereby decreasing ineffective erythropoiesis. This study aims to investigate the role of calpain and its inhibitors, calpastatin, as modulators of variable severity of the thalassemia disease. Erythroblasts were generated *in vitro* from peripheral CD34⁺ blood cells from patients who have mild and severe clinical symptoms. Calpain activity, protein- and mRNA amounts, and the intracellular localization of μ -calpain and calpastatin were examined. The results illustrate that calpain activity is significantly elevated associated with an increase of calpain protein amounts, whereas tissue-type calpastatin protein amounts are significantly decreased in patients with mild compared to patients with severe clinical symptoms. However, mRNA amounts are not significantly different between the patient groups. Intracellular activation of μ -calpain is confirmed by diffusion of calpastatin into the cytosolic compartment of thalassemic cells.

Altogether our findings reveal that the increase in calpain activity may accelerate the degradation rate of unstable α -globins in erythroid precursors of patients with mild clinical symptoms, leading to reduced precipitation and resulting in less severe pathology.

27. MICRORNA 210 EXPRESSION IN THALASSEMIC ERYTHROPOIESIS

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Ineffective erythropoiesis, the major cause of anemia in α -thalassemia, was demonstrated to be due to apoptosis of erythroid progenitor cells in the bone marrow. MicroRNAs (miRNAs) are negative regulators of gene expression that play an important role in development, differentiation and cell death. A unique composition of miRNAs in each hematopoietic progenitor cell type suggested that these miRNAs determine the final output of gene expression and mediated control of normal human hematopoiesis and lineage commitment. In this study expression pattern of miR-210, which has been shown to be expressed during normal erythropoiesis and involved in apoptosis, was examined in thalassemic erythroid cells from α -thalassemia/HbE patients and α -thalassemic mice. The measurement of apoptotic erythroid progenitor cells by annexin V assay revealed the higher phosphatidylserine exposed basophilic erythroblasts in α -thalassemic bone marrow than that of normal. Up-regulated expression pattern of miR-210 was observed, especially in basophilic erythroblast derived from peripheral blood CD34+ cells of α -thalassemia/Hb E patient. This is in consistence with the finding in α -thalassemic mouse model, which showed the significantly higher level of miR-210 expression in thalassemic immature erythroid cells when compared to that of wild type mice. Our results suggested that early erythroid progenitors in α -thalassemia/Hb E patients and α -thalassemic mice have a dysregulated miR-210 expression program. Analysis of microRNAs is a relevant approach to determine abnormalities of erythropoiesis.

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28. EXPRESSION OF GLOBIN GENES IN DIFFERENT THALASSEMIA DISORDERS

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The thalassemia, characterized by reduced or absent production of one type of globins in the hemoglobin molecule, leads to the imbalanced α /non α -globin chains. A range of clinical severities in both α - and β -thalassemia syndromes varied from nearly asymptomatic to transfusion-dependent. Measurement of α /non α -globin synthesis ratio *in vitro*, which normally used radioactive labeled amino acid is the direct method to determine the imbalanced globin chain. In this study we investigated the clinical heterogeneity and the levels of globin gene expression in various genotypes of thalassemia. Quantitation of α -, β - and δ -globin mRNAs was carried out from reticulocyte by multiplex RT-qPCR, which is simple, fast and no radioactive material involved. The result demonstrated that the α /non α -globin mRNA ratio is a good indicator of disease severity in different thalassemia disorders. We also studied pre-mRNA splicing ratio of HbE, a G to A mutation in codon 26 of β -globin gene. This abnormal gene activates a cryptic 5' splice site in codon 25 leading to a reduction of correctly spliced β^E -globin mRNA and consequently β^+ -thalassemia. The level of correctly and aberrantly spliced β^E -globin mRNAs was measured by allele specific RT-qPCR. The result showed that the correctly/aberrantly spliced β^E -globin mRNA ratio in 30% of mild cases was higher than that of the severe patients. This study indicated that splicing process of β^E -globin pre-mRNA is differed among α -thalassemia/HbE patients and may serve as one modifying factor for disease severity.

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29. ASSOCIATION of XMN I POLYMORPHISM AND HEMOGLOBIN E HAPLOTYPES ON POST-NATAL GAMMA GLOBIN GENE EXPRESSION in homozygous HEMOGLOBIN e

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Beside producing abnormal variant; Hemoglobin E (HbE), the G→A substitution in codon 26 (Glu→Lys) of the γ -globin gene (γ^E) could also produce δ^+ thalassemia due to decreased functional HbE-mRNA, secondary to alternative splicing mechanism. However, the clinical phenotype in homozygous Hb E (Hb EE) is rather asymptomatic with very mild anemia. In contrast, patients with HbE/ δ^+ thalassemia have a more diverse clinical phenotype from transfusion-dependent to very mild disease. Variation of post-natal γ globin expression and HbF production in these patients was thought to be responsible for their clinical heterogeneity by reducing globin imbalance. Through erythroid development, the γ globin expression was regulated by interactions between *cis*-acting sequences within the γ globin cluster and *trans*-acting factors such as BCL-11A, cMYB and TOX. The most significant genetic factor in *cis* associated with high HbF is *Xmn I* polymorphism located at -158 upstream to the γ^E globin genes. In a recent study using a more refined SNP analysis of the γ globin gene cluster in HbE/ δ^+ thalassemia, has shown that there was no other variant elsewhere which has a comparable level of association with that of *Xmn I* site and the T allele (*Xmn I*; +) was nearly always in *cis* with the HbE alleles (Ma Q. et al, 2007). To further explore the role of *cis*-acting sequences on Hb F production, we analysed 80 Hb EE in which 15% of them (n=12) had high level of Hb F (average 10.5%; range 5.8-14.3%), while the rest had 100% HbE. Analysis for standard γ globin haplotypes as described by Orkin; γ^E , *HindII*; γ^E , *HindIII*; γ^E , *HindIII*; 5' and 3' γ^E , *HindII*; 5' γ^E , *Avall*; 3' γ^E , *Hinfl* including *Xmnl* was performed by RFLP. Using the Phase-Standard analysis, eight different γ globin haplotypes associated with HbE alleles have been constructed with two novel ones; ----+ and ++-+-. Three major haplotypes; III (60.4%), V (24.02%) and IV (9.74%) were accounted for the majority of HbE alleles and they were linked with *Xmnl* +, - and + respectively. Therefore only 71.43% of HbE allele is linked with the T allele of *Xmnl*. In 6 pediatric cases (under 15 yrs) with high HbF, our subsequent study showed a decreased or absent of HbF on their follow up. While in adult cases, their High HbF remained persistent excluding temporary hematopoietic stress. Interestingly, there was no significant association between specific γ globin haplotypes and *Xmnl* polymorphism in these individuals compared to the rest. Even though the average alkali F levels were albeit increased in Hb EE with *Xmnl*; +/- compared to +/- but it was statistically insignificant. Together, our data suggest that HbF production found in these rare Hb EE individuals might be mainly controlled by *trans*-acting mechanism and they may provide a novel, rather less complicated natural model for further study on molecular mechanism controlling γ globin expression.

30. EVALUATION OF ADVERSE EVENTS DUE TO DEFERASIROX; A NOVEL ORAL IRON CHELATOR, IN 79 THAI PEDIATRIC PATIENTS WITH BETA-THALASSEMIA

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A novel oral iron chelator; deferasirox (Exjade[®]) provides a new hope for better chelation therapy in thalassemia patients owing to its once daily dose administration. Data from randomized controls studies indicated that deferasirox was associated with clinically manageable tolerability profile. However, such adverse events data are limited in pediatric patients and mainly derived from Caucasian population. To evaluate the adverse events of deferasirox, we prospectively collected clinical data of adverse events in pediatric β -thalassaemia patients whom received deferasirox for treatment of iron overload. A standard protocol to routinely evaluate their hematological, renal, liver and cardiac profiles was set and followed in all patients. Audiologic and ophthalmologic evaluations were performed annually. Seventy nine patients (33 male: 41.7%) were included with the mean age of 12.3 \pm 3.7 years (range 4-19 years). The median follow-up time was 12.3 mths (range 2.7-26.5). The average dosage of deferasirox was 22.6 \pm 4.2 mg/kg/d (range 16-30). Forty nine patients were well tolerated without any adverse events (62%). Most common side effects included rash (17.7%); usually mild, transient and resolved spontaneously, gastrointestinal complications; abdominal pain (8.9%), increase stool frequency (5.1%), nausea/vomiting (2.5%) and anorexia (2.5%). Occasional proteinuria was detected in 9.8% and this was resolved with drug interruption. We also found 2 UTI episodes in one patient, one occult hematuria (1.3%) and one hemorrhagic-cystitis (1.3%). Transient neutropenia (ANC <1500 cells/mm³) were found in 2 patients (2.5%), both were completely recovered and might not be related to the drug. Interestingly, we found 2 serious adverse events that have never been reported before; 1 optic neuritis and 1 pancreatitis, deferasirox was terminated after these complications were detected. However, the relationship between the treatment and these adverse events remains unclear. Transient skin rash was the

most common adverse event, followed by manageable gastrointestinal complications. A significant number of cases with proteinuria in addition to previously reported patients with non-progressive increase creatinine clearance in our study have warranted a further study on pathogenesis of possible renal complication due to deferasirox.

31. HETEROGENEOUS SPECTRUM OF MOLECULAR BASIS OF ALPHA-THALASSEMIA IN THAILAND; A GENOTYPE ANALYSIS IN 500 PATIENTS WITH Hb H DISEASE

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HbH disease is a common, moderately severe form of alpha thalassemia which occurs throughout Southeast Asia, the Mediterranean and the Middle East. In Thailand where nearly 25% of the population are carriers of thalassemia, we expect 7,000 new cases of HbH disease every year. Previous studies have shown the molecular basis of HbH disease in Thailand was mainly caused by deletions of the alpha cluster (deletional Hb H disease; $\alpha\alpha^{-}$). Nevertheless a substantial number of patients have non-deletional forms of Hb H disease ($\alpha\alpha^{-T}$), most often associated with HbH/Constant Spring ($\alpha\alpha^{-CS}$). In many surveys, HbCS, a termination codon mutation (TAA CAA), has been identified as the most common non-deletional type of thalassemia in Thailand. However, there is increasing evidence that other non-deletional thalassemia mutations might have been under diagnosed due to diagnostic limitation in the past. Recently, we have successfully characterised nearly all thalassemia alleles (999 from 1,000 alleles, 99.9%) from 500 Thai pediatric patients with HbH disease using DNA analysis. Deletional types of Hb H disease were found in 213 cases (42.6%). Their genotypes are; 1). $\alpha\alpha^{-SEA/3.7}$ (n=199, 39.8%) 2). $\alpha\alpha^{-SEA/4.2}$ (n=10, 2%) 3) $\alpha\alpha^{-THAI/3.7}$ (n=4, 0.8%). As previously noted, in this study, Hb H/CS was the most common non-deletional mutant identified; $\alpha\alpha^{-SEA/CS}$ (n=247, 49.4%) and $\alpha\alpha^{-THAI/CS}$ (n=2, 0.4%). In addition, several non-deletional mutations were also found, all interact with SEA deletion including; HbH/Hb Paksé ($\alpha\alpha^{-SEA/PS}$, termination codon, TAA TAT, n=28; 5.6%), Hb H/Pak Num Po ($\alpha\alpha^{-SEA/PNP}$, codon132 with T insertion, n=3; 0.6%) and HbH/Hb Quong Sze ($\alpha\alpha^{-SEA/QS}$, codon 125, CTG CCG), n=2; 0.4%). In addition, rare initiation codon (ATG A-G), a polyA (AATA-) and Hb Westmead (codon 122, CAC CAG) mutations were also found (n=1 each). Interestingly, a novel codon 32 mutation (ATG AAG) has been identified for the first time in this study causing α^+ thalassemia. In one case, despite analysis of the alpha globin genes and their regulatory elements, the cause of alpha thalassaemia remains unknown. In general, the haematological and clinical phenotype of patients with non-deletional forms of HbH disease is more severe than those with deletional forms of HbH disease. In summary, our study shows that molecular basis of HbH disease in Asia is more heterogeneous than previous thought and the genotype analysis described here provides an effective protocol for obtaining a definite molecular diagnosis. This, in turn, will allow us to explore in detail the relationship between genotype and phenotype in this common form of thalassemia.

32. HIGH RATES OF CARBOHYDRATE ABNORMALITIES BY OGTT IN ASYMPTOMATIC PATIENTS WITH BETA THALASSEMIA MAJOR (TM)

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Introduction: Diabetes mellitus (DM) is a known complication of beta TM. The prevalence of impaired glucose tolerance (IGT) in TM and progression to DM are unknown. The aim of this study was to determine the prevalence of glucose abnormalities based on oral glucose tolerance (OGTT) in subjects without clinical symptoms of DM, and describe associated factors. Methods: Retrospective chart review of all TM patients at Weill Cornell Medical College who had OGTTs and no clinical evidence of DM. Age began Deferoxamine (DFO), OGTT results, BMI, Tanner stage, and ferritin level were recorded.

Results: Of the 35 subjects, 13 (37%) had normal OGTT, 15 (43%) had IGT and 7 (20%) had DM. 80% of glucose abnormalities were based on the 2hr OGTT result and not on fasting values. The youngest age of IGT was 10y and DM 13y. 18 subjects had multiple OGTTs (up to 4 tests over the span of 7.5y). Progression of glucose abnormalities was variable. Of the 4 patients with DM at the initial test, 2 reversed to IGT on follow up. 3 subjects with IGT progressed to DM. Subjects with IGT at the first visit had significantly higher ferritin levels than those with normal glucose tolerance (NGT) (3058 vs. 1319 ng/mL; p<.05). Subjects with any history of IGT began DFO significantly later than those with NGT

(7.9 vs. 4.6 years; $p < .05$).

Conclusions: High rates of carbohydrate intolerance (i.e. IGT or DM) were found by OGTTs in otherwise asymptomatic TM patients. They were associated with ferritin levels and age of start of DFO treatment, suggesting that iron overload is involved in the pathogenesis. The long term significance of these abnormalities in the aging thalassemia patients, as well as interventions to prevent the development of clinical DM merit further studies.

33. Augmentation of hematopoietic engraftment without graft versus host disease by “add-back” of photochemically treated T lymphocytes in mismatched cord blood transplantation

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Cord blood transplantation (CBT) is being increasingly used as a source of stem cells to cure malignant and non-malignant diseases. Unrelated CBT is associated with higher rates of graft rejection and autologous reconstitution, in part due to the finite stem cell content of the CB unit. CB is also inherently deficient in effector T cells which play an important role in engraftment secondary to the secretion of cytokines essential for stem cell homing and niche space creation. Several strategies to improve engraftment in CBT, including co-transplantation of adjuvant immunomodulatory cell population, have encountered limited success, especially, in clinical setting. Here, we investigated a novel method to improve engraftment kinetics of cord blood transplantation, without the antecedent graft versus host disease, by the co-infusion of photochemically (psoralen) treated mature donor T lymphocytes in a major histocompatibility complex (MHC) [H2- haplotype] mismatched murine model.

We analyzed the donor chimerism, graft versus host disease (GVHD), hematopoietic engraftment and long-term survival in H2 haplotype disparate (C57BL/6 AKR) mice model after stem cell transplantation. Three different experimental groups were transplanted after sublethal radiation. Group 1 received allogeneic full term newborn peripheral blood (FNPB), group 2 was transplanted with FNPB and unmanipulated donor T cells, and group 3 was transplanted with FNPB and psoralen (S-59) treated donor T cells.

Full donor chimerism and minimal GVHD was observed in group 3 after co-transplantation of 3×10^6 nucleated cells (NC) and 3×10^6 S-59 treated T cells per mouse. The engraftment rates were significantly higher in group 3 (75%) relative to group 1 (12.5%), at 3 fold lower NC dose with addition of S-59 treated lymphocytes at escalated (9×10^6) doses ($p=0.007$). The long-term survival in group 3 (100%) was superior relative to group 1 and 2 (97% and 50% respectively). Similar engraftment obtained in group 2, at equivalent nucleated and T cell doses, was off-set by inferior survival rates secondary to severe GVHD. Durable engraftment with donor MHC class 1^{high}-c-kit⁺/c-kit low and c-kit <low cells was noted in both the bone marrow and spleen of the engrafted group 3 recipient mice. Donor derived myeloid, B cells and T cells were noted in the spleen and bone marrow by day 30, in the group receiving FNPB and S-59 treated T cells. Recovery of hematological parameters in group 3 mice was delayed and occurred between days 50-60 following transplantation.

These results demonstrate the ability of donor psoralen treated T lymphocytes to improve cord blood engraftment kinetics across disparate H2 haplotype. Co-transplantation of psoralen treated lymphocytes with CB cells also lead to durable engraftment of donor MHC high/c-kit⁺ cells in the bone marrow and splenic compartment leading to delayed but complete hematopoietic engraftment. The reduced GVHD risk and improved long term survival observed in this murine model suggests that a similar approach may be considered as a potent immuno-modulatory tool in cord blood transplantation.

34. IRON OVERLOAD MODIFIES THE INNATE IMMUNE RESPONSE IN THALASSEMIA MAJOR

Patrick B Walter, PhD, Paul Harmatz, MD, Annie Higa, BS, Nancy Sweeters, RN, PNT, Ashutosh Lal, MD, David W. Killilea, PhD, Elliott Vichinsky, MD, Children's Hospital & Research Center Oakland, Oakland, CA

Infection is the second most common cause of death in thalassemia. The innate immune system provides a first line of defense against infection, which relies on pattern recognition receptors (PRRs) that are specific to microbial pathogens. One class of PRR called the toll-like receptors (TLRs) are important for transducing the signal for bacterial lipopolysaccharide (LPS), resulting not only in cytokine production, but also in managing extracellular iron levels through production of neutrophil gelatinase associated lipocalin (NGAL). However, the exact role that NGAL plays and the expression level of PRRs in thalassemia are unknown. Thus, the goal of these studies is to investigate the relationship of iron overload to the innate immune cell expression of PRRs and NGAL in thalassemia. Fifteen transfusion dependent

thalassemia patients participating in the combination trial of deferasirox and deferoxamine were enrolled (Novartis sponsored C1CL670AUS24T). Fasting blood samples were obtained at baseline and at 6 and 12 months on study. Five healthy controls were also enrolled. Fresh monocytes were isolated using antibody-linked magnetic microbeads and analyzed by flow cytometry. Our preliminary results show that baseline expression of TLR4 was reduced in monocytes from patients with thalassemia compared to healthy controls. Moreover, the plasma levels of NGAL were elevated in patients with thalassemia compared to healthy controls. These results suggest that chronic iron burden in thalassemia can modulate the innate immune response in thalassemia, possibly preventing the uptake of excess iron by pathogenic bacteria.

35. SEVERE THALASSEMIA INTERMEDIA RESULTING FROM COINHERITANCE OF IVSI-110 G>A BETA GENE MUTATION AND DUPLICATION OF α -GLOBIN GENE CLUSTER IN HOMOZYGOSIS

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The clinical severity of thalassemia intermedia depends on the degree of α /non- α -chains imbalance, as the coinheritance of excessive α -globin gene production with a β -globin gene mutation. This mechanism was found in an Italian family where thalassemia intermedia apparently segregates as a dominant form. The father showed a chronic hemolytic anemia (Hb 7-8 g/dL) not transfusion dependent, jaundice, splenomegaly and leg ulcers. The mother has a normal hematological pattern. Two sons showed more severe clinical manifestations; they underwent splenectomy without any benefit and afterwards they became transfusion dependent. The hemoglobin analysis revealed that the father and the sons were heterozygotes for the beta mutation IVSI-110 G>A. MLPA analysis of the alpha-globin gene cluster disclosed a full duplication of the alpha-globin locus, spanning a 175 kb from the telomere to the 3'HVR downstream of the alpha-globin gene and including the upstream regulatory element HS-40. This rearrangement increases the number of the active alpha-globin genes in cis from 2 to 4, founded in heterozygosis in both parents and in homozygosis in both sons. In the father the 6 alpha-globin genes led to increased synthesis of alpha-chains; the coinheritance with a beta-thalassemia mutation causes a moderate/severe thalassemia intermedia phenotype. The presence of 8 alpha-globin genes in the sons exacerbates the clinical phenotype. Our previous experience suggests that splenectomy in patients with an excess of alpha chain production is inconvenient.

POSTER PRESENTERS – Friday, October 23

1. Agouzal Mouna
2. Anabel Arends
3. Janice R Beatty
4. Lisa Bjorkelo
5. Caterina Borgna-Pignatti
6. Susan Carson
7. Christina Chin
8. Gina Cioffi
9. Gina Cioffi
10. Patricia Giardina
11. Derchi Giorgio
12. Dru Foote
13. Ellen B. Fung
14. Roland Fischer
15. Mona Hamdy
16. Kallistheni Farmaki
17. Tatjana Kilo
18. Dorothy Kleinert
19. Robert C. Yamashita
20. Carsten W. Lederer
21. Robert Liem
22. Rashid Merchant
23. Andreas Michos
24. Zahra Pakbaz
25. Inusha Panigrahi
26. Amy Sobota
27. Isabelle Thuret
28. Rashid Merchant
29. Vip Viprakit
30. Vip Viprakit
31. Jin Yamamura
32. Robert Yamashita
33. Leila J. Noetzli

1. BETA THALASSEMIA MAJOR: THE MOROCCAN EXPERIENCE

Agouzal M, M.Sc.¹, Quyoun A, Pr.Sc.¹, Khattab M, Pr.², ¹Ibn Tofail University, ²Center for thalassemia, Rabat, Morocco

Thalassemia has been described originally around the Mediterranean sea. Our main objective is to show that Morocco deals with the same situation as well. We aim also to detect risk factors of thalassemia.

It is a retrospective (descriptive and causative) study. It has been done in the hemato-oncology service that is in charge of patients with beta thalassemia. These patients are alive and registered as receiving blood transfusions. Sample size is 104. Demographics, clinical and family data were collected. Statistics were done in the biological essays laboratory in Kenitra. Study duration was six months. Age bracket is between 5 and 10 years. Males are predominant. 68% of the patients have a family history. Sickle cell anemia is associated to beta thalassemia among 34% of the patients. 67% of patients with beta thalassemia major are chelated as only 14% has a ferritinemia rate <1000 ng/ml. Among these patients, 74% are indigent. Origin of patients is mostly from the axis Rabat-Sale-Kenitra. Risk factors for occurrence of beta thalassemia are blood group A and consanguinity.

These results confirm that the disease is a reality in our country. Scientists and parents strongly recommend a public health policy towards this disease.

2. MOLECULAR STUDIES OF BETA-THALASSEMIA MUTATIONS IN VENEZUELAN POPULATION AND THEIR LINKAGE TO BETA GLOBIN GENE HAPLOTYPES

Martha Bravo-Urquiola, PhD¹, Anabel Arends, M.D, PhD¹, Gilberto Gomez, B.Sc¹, Silvia Montilla, B.Sc¹, Marycarmen Chacin, B.Sc¹, Odalís García, B.Sc¹, Dalia Velásquez, M.D¹, Nathalie Gerard, B.Sc², Omar Castillo, PhD³ and Rajagopal Krishnamoorthy, PhD². ¹Laboratorio de Hemoglobinas Anormales, Hospital Universitario de Caracas and Instituto Anatómico José Izquierdo, UCV, Caracas, Venezuela. ²INSERM U763, Hospital Robert Debre, Paris, France. ³Universidad de Carabobo, Venezuela

In order to establish the frequency and spectrum of Thalassemia (Thal) mutation in Venezuelan population, a total of 127 unrelated patients were analyzed by polymerase chain reaction followed by reverse dot blot (RDB), ARMS-PCR, DGGE and direct sequencing. Fourteen different mutations were identified accounting for 92% of the alleles studied, revealing a high genetic heterogeneity at the beta locus in this population. The most frequent mutations were: CD39 (C→T) 34.5%, IVS1-1 (G→A) 11.3%, IVS1-6 (T→C) 6.7%, IVS1-110 (G→A) 6.7% and IVSII-849 (A→G) 6.7% and the less common mutations were -29 (A→G) 5.3%, -88 (C→T) 5.3%, IVS1-5 (G→A) 3.8%, Del 1.39 Kb 3.0%, IVSII-1 (G→A) 3.0%, -86 (G→C) 2.3%, IVSII-1 (G→T) 1.5%, CD41/42 (-CTTT) 1.5% and IVSII-745 (C→G) 0.8%. The beta globin gene cluster haplotypes were determined in 70 beta thalassaemic chromosomes using the PCR-RFLP technique. We found different haplotypes linked to the beta thalassaemia chromosomes and each mutation was associated with one or two specific haplotype. The spectrum of beta thalassemia mutation and the analysis of haplotypes of chromosomes bearing each beta thalassemia mutation suggested that the origin of beta thalassemia in Venezuela is Mediterranean and African and that the spread of these mutations were reflected by the historical records. This work was supported by grants from Fonacit S1-2002000539, G-2005000373, MC-2007001066. Ecos-Nord PI-2005000758, CDCH PI-090064512006, CDCH PI-0973022008.

3. STEP UP: FOSTERING ADULT SKILLS IN ADOLESCENT THALASSEMIA PATIENTS

Janice R Beatty, BSN, Diane M Calamaras, MSN, CPNP, Erin B Gordon, MSW, LCSW, Kristin B Culp, MS, CGC, Stephanie A Pelligra, BS, A Kyle Mack, MD, Alexis A Thompson, MD, Division of Hematology/Oncology/SCT, Children's Memorial Hospital, Chicago, IL

The transition process to an adult-oriented model of health care is one of the most difficult challenges facing pediatric providers caring for adolescents with thalassemia. Recent advances in the treatment of thalassemia have extended the lifespan of this population. The Children's Memorial Hospital Thalassemia Program cares for over 100 children and adults with thalassemia with a median age of 14 years (range 1-58). We hypothesized that a systematic, multidisciplinary approach to promote self-efficacy and enhance thalassemia education are required to improve the process of transitioning to adulthood. A transition protocol and support materials have now been tailored to the needs of the adolescent with thalassemia. They form the basis of a project which we have called, "STEP UP: Managing Thalassemia as an Adult: A Transition Guide". A brief self test was designed to assess each patient's current knowledge of issues related to thalassemia as well the impact of educational interventions. Core competencies for our targeted patient population (ages 14-18) have been identified and are assessed using

an interdisciplinary checklist which is reviewed with the adolescent and their parents at each comprehensive visit. A binder was created which contains contact information, comprehensive patient care plan, health insurance/disability information, thalassemia resources and educational materials. The Step Up Program was launched in July 2008. To date, 10 patients have been enrolled. Metrics will include compliance self-reporting, serum ferritin values, % completing high school, employment and insurance status. Analysis of baseline data shows knowledge deficits. We anticipate that formalizing the process to foster self-efficacy in adolescents with thalassemia will ensure success in the transition to becoming an independent adult.

4. THALASSEMIA SELF-LEARNING MODULE FOR NURSES

Lisa Bjorkelo RN, Marie Martin RN, Children's Hospital of Philadelphia, Philadelphia, PA

At the Children's Hospital of Philadelphia, forty pediatric and adult Thalassemia patients receive their chronic transfusion therapy in the Oncology Clinic Day Hospital. The majority of nurses in the oncology clinic are specialized and certified in pediatric oncology. The nursing staff is quite knowledgeable about providing safe nursing care to patients receiving blood transfusions. However, there was a knowledge deficit related to Thalassemia pathophysiology, treatment and side effects, particularly related to adult patients.

A Thalassemia Self-Learning Module (SLM) was developed so that staff nurses could provide more comprehensive quality care to the Thalassemia population in the day hospital. The information in the SLM was initially presented to eleven clinic nurses with an average of 9.8 years of nursing experience. Two pretests were given prior to the viewing the SLM which ascertained the nurses general knowledge about Thalassemia as well as perceived comfort level in providing nursing care to pediatric and adult Thalassemia patients. The same tests were repeated after the SLM was viewed with significant increases in scores. Comfort level increased from 2.6 to 3.9 (on a 1-5 scale). Thalassemia general knowledge test scores increased from 47% to 99%.

The Thalassemia SLM is an effective tool for educating staff nurses about Thalassemia. It also increased the nurses perceived comfort level in providing nursing care to both children and adults with Thalassemia. The SLM has now become part of the orientation process for new nurses in the day hospital.

5. UPDATE OF SURVIVAL OF AN ITALIAN POPULATION OF THALASSEMIA PATIENTS

Caterina Borgna-Pignatti, MD¹, Assunta Santullo MD¹, Stefano Volpato MD², Dept of Clinical and Experimental Medicine (Pediatrics¹ and Internal Medicine²) University of Ferrara, for the 7Center Italian Survival Study*

Since 1983 we have reported the survival and causes of death in a population of Italian patients with thalassemia major. The update performed on December 2008, 10 years after the last published report, is described here. Follow-up data were available for 949/997 patients. Median follow-up was 26 years, the oldest patient being 48 years old. Overall 229 patients had died, 43 of whom since 01/01/2000. 151 pts died of cardiac causes, 20 since the last follow-up, 14 died of infection, 8 of cancer, 3 of liver disease, 10 patients died of unknown cause. Over 90% of the patients born after 1975 (when chelation with deferoxamine became widely available) were alive at follow-up. Females continue to survive significantly longer than males, the difference being due to a lower rate of cardiac disease in females.

Ferritin levels were significantly higher in patients with heart disease, and patients who died.

We conclude that survival in Italian patients with thalassemia major is excellent, especially for those who had the benefit of chelation since the first decade of life. The first cause of death continues to be cardiac disease.

* F. Bonetti, University of Pavia, M.D.Cappellini MD, E.D'Angelo, University of Milano, G.C. Del Vecchio, University of Bari, G.L. Forni, Galliera Hospital, Genova, M.R. Gamberini, Sant'Anna Hospital, Ferrara, A. Piga University of Torino, M.A. Romeo University of Catania.

6. TREATMENT OF TWO THALASSEMIA PATIENTS WITH LONG-TERM RITUXIMAB THERAPY FOR AUTOIMMUNE HEMOLYTIC ANEMIA: A NOVEL APPROACH

Carson, Susan, CPNP¹, Nord, Anne RN¹, Hofstra, Thomas MD¹, Wood, John MD², Coates, Thomas MD¹, ¹Childrens Hospital Los Angeles, Division of Hematology/Oncology, ²Childrens Hospital Los Angeles, Division of Cardiology

Transfusions are the mainstay of treatment for patients with thalassemia. Over a lifetime of chronic transfusions, however, many develop red cell antibodies and clinically significant hemolysis, which is traditionally treated with steroids or other immune modulating therapies. Rituximab is being used increasingly for various autoimmune disorders, but there is very little literature describing its use in thalassemia patients with alloimmunization. Published reports describe treatment with a short course of rituximab.

We describe the therapy and clinical outcomes for 2 patients who had very significant antibody levels that failed to respond to traditional therapy. Initial responses were attained with a short course of rituximab, but neither patient maintained a prolonged response. Both demonstrated clinically significant antibody recurrence and hemolysis. Subsequently both patients were re-induced with weekly rituximab. To reduce the likelihood of adverse effects while maintaining a response, rituximab therapy was continued with gradual lengthening of the intervals between treatments. Both patients currently receive rituximab every 10-12 weeks, having continued on this novel “maintenance rituximab regimen” for several years without recurrence of their clinically significant antibodies and without reported side effects. This therapy merits further study, and may hold promise for patients with severe alloimmunization from repeated transfusions.

7. A PATIENT’S VIEW AND SUGGESTIONS FOR FURTHER RESEARCH

Christina Chin, Pharm.D. Terry Chin, MD, Penny Chin, R.N., Irvine and Yorba Linda, CA

From the prospective of a patient, questions regarding the best mode of therapy continue to exist. Obtaining adequate care as an adult is another issue and topic. This following is an account of one patient with questions suggesting possible studies to confirm her observations.

The diagnosis of beta-thalassemia was made at 8 mos of age and regular (every 4 wks) RBC transfusions were instituted. Measurements of 24 hr iron secretion in both stool and urine before and after subcutaneous administration of desferoximine (Desferal[®] or Df) indicated sufficient iron elimination at age 13 mos. Therefore, a regular program of daily subcutaneous (sc) injection of Df was instituted for 17 years. For the past 14 years, a program of intensive intravenous (iv) Df concomitant with the regular RBBC transfusion for 72 hours without daily sc Df was instituted. This was due to my observation that most of the urinary excretion of iron appeared to occur at the time of RBC transfusion, continuing for about 1-2 weeks afterwards, and only with iv Df administration. I have been very compliant with both iv and sc Df.. This approach appeared to be adequate for me and allowed for a more normal life-style. The placement of a Port-a-Cath at 17 years of age resulted in improvement in obtaining iv assess for RBC transfusions and also allowed home iv Df. My first Port-a-Cath lasted for 14 years. I still have my spleen; and do not have to worry about the increased risk of infection which would have been an outcome of that surgery. At 31 years of age, my ferritin level has been less than 200 µg/dL and liver enzymes normal. However, 6 years ago measurement of cardiac iron by T2* was abnormal and my ejection fraction was 45%. Increasing the frequency of iv Df resulted in only minimal improvement. However, deferiprone (Ferriprox[®]) resulted in normalization of both T2* and echocardiogram. This indicates the need to consider cardiac iron accumulation even with normal hepatic function and ferritin levels. My life’s example suggests a need for improvement in evidence-based treatment.

8. PATIENT PERCEPTIONS OF THE VALUE OF THALASSEMIA TREATMENT CENTERS (TTC)

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Background: Thalassemia Treatment Centers (TTC) provide comprehensive services for patients with thalassemia, including, not only transfusions, but assessment, prevention and treatment services for complex secondary conditions related to iron overload. The perceived value of these services has not been assessed from the patient/family point of view. In this CDC-funded project, we address this lack of data through formative research to learn the perceived value of the federally-funded TTCs. **Methods:** 12 Telephone focus groups and 11 individual interviews were conducted with a total of 78 participants (patients and caregivers). **Results and Discussion:** 38% of the participants were seen at a TTC, 45% were seen at a local hospital and have never gone to a TTC for an evaluation, and 13% were seen at a local hospital but visited a TTC at least once every two years. Focus group participants were not segmented by TTC use, however, participants talked about their TTC experiences. Perceived benefits included access to experts with years of experience in thalassemia care who are willing to discuss care options; access to state of the art evaluation technology and tests; access to a variety of specialists working in a team with their hematologist; and the opportunity to meet other people with thalassemia. Some participants expressed frustration in finding high quality of care in a convenient setting without long commutes. Others felt traveling for an annual visit was a good investment. Increasing access to TTC services should be explored.

9. EXPLORING BARRIERS AND FACILITATORS TO TREATMENT ADHERENCE AMONG PEOPLE WITH THALASSEMIA AND THEIR CAREGIVERS THROUGH FOCUS GROUPS

Gina Cioffi, JD Bonnie Bates, MA Craig Butler, MA , Kathleen Durst, MA, LMSW , Karina Jimenez-Donovan, MSW , Ellis Neufeld, MD, PhD Sally McAlister Jessica Palmerini, MPA , Eileen Scott , Gretchen Simmons, MPH, CHES , Alexis A. Thompson, MPH, MD , Rikki Welch, MA

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Background: Consistent adherence to prescribed treatment regimens in thalassemia will lead to improved health outcomes. This is true not only for primary treatment of anemia, but also for secondary complications, notably iron overload, for which treatment can be burdensome. **Methods:** Formative research, in the form of telephone focus groups and individual interviews, was conducted to assess the attitudes, knowledge, beliefs and behaviors among people with severe (transfusion-dependent) and mildly/moderately affected thalassemia to develop a greater insight into why people with thalassemia and their caregivers choose to adhere or not to prescribed treatment regimens. Areas explored included: adherence to treatment; prevention behaviors; self-management of thalassemia; knowledge, beliefs and practices; and the perceived value of Thalassemia Treatment Centers (TTCs). **Results and Discussion:** A total of 78 participants (patients and caregivers) participated in 12 focus groups and 11 individual interviews. Participants generally stated they adhered to treatment regimens; they identified common barriers to adhering to treatment; and understood severe health consequences of non-adherence. Recommendations included increasing dissemination of health information; providing opportunities for people with thalassemia to share success stories and strategies to cope with the challenges of thalassemia; fostering communication within the thalassemia community; promoting successful transition for youth to adulthood; and reviewing the value of TTCs. These results will

10. PSYCHOLOGICAL COPING IN ADULTS WITH THALASSEMIA

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This study investigates how patients with thalassemia, a chronic and potentially life-threatening disease, cope with the chronic stress of treatment and maintenance of their illness.

Thirty-eight thalassemia patients completed self-report questionnaires about their mental and physical health and social relationships and participated in a structured clinical interview (SCID) to assess Major Depression, Generalized Anxiety, and Post Traumatic Stress Disorder. Physician ratings of patients' compliance with medical treatment were recorded. Patients, as a group, appeared to be functioning well. Nearly 3/4 had completed college and 2/3 were employed, most full-time. Five of 38 patients met criteria for at least one of the psychiatric disorders assessed on the structured clinical interview, a prevalence rate comparable to that of the normal population. Women adapted significantly better than men, rating themselves higher on coping skills, psychological health, and self-confidence. A strong psychological defense mechanism (self-enhancement) was associated with less distress and fewer psychological symptoms. Results suggest that the majority of patients with thalassemia can cope well with their illness, but that future interventions for those who are struggling may be most useful if focused on developing enhanced self-esteem.

11. PULMONARY HYPERTENSION IN THALASSEMIA MAJOR: AN ITALIAN MULTICENTRIC STUDY

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The prevalence of pulmonary hypertension (PAH) in Thalassemia Major (TM) is reported to be 10-75% depending on age, therapy and coexisting LV dysfunction (LVD). To determine whether PAH can occur in TM in absence of overt LVD, we studied 368 pts (49% males, mean age 33,7 yrs, range 16-58 yrs). In all pts left ventricular systolic dysfunction (EF< 50%) and clinically overt heart failure were excluded. In this population, PAH was prospectively defined as mild with tricuspid regurgitant jet velocity (TRV) of at least 2.5 m/sec and severe with a TRV> 2,8 m/sec. Severe PAH was observed in 30 pts, mild PAH occurs in 55 pts. In the remaining 201 patients (70,2%), TRV was in the normal range. In the whole group of TM pts, the prevalence of significant PAH was 9.8 %. Our data support a significant presence of PAH in TM pts even though well treated and without overt LV dysfunction. In addition we found an higher prevalence of severe PAH in older, splenectomized patients and also a significant inverse correlation between increased PAH and EF%, suggesting an interaction of these factors in the disease progression in TM.

12. PAIN AS AN EMERGENT ISSUE IN THALASSEMIA

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The aim of this study was to examine the prevalence, severity, and effects of pain in the Thalassemia Clinical Research Network (TCRN). 245 adults/adolescents and 99 children (87% transfusion-dependent) enrolled in the Thalassemia Longitudinal Cohort (TLC) were assessed using the SF-36v2 or the Children's Health Questionnaire PF-28. 68% of adults/adolescents reported bodily pain in the past 4 weeks, with 27% reporting at least moderate pain. Parents reported pain in 58% of children, with only 10% reporting pain fairly often. When compared to the general population, there were no significant differences in pain in thalassemia children. However, in adults/adolescents, pain increased significantly with age (p<0.001). Patients older than 35 years of age experienced significantly more pain than the general population. When quality of life is evaluated by amount of reported pain there was a significant decrease in quality of life in all domains with increasing pain (p<0.001), with a higher correlation with the physical component (r=0.80) than the mental component (r=0.32). Pain was also significantly correlated with increased anxiety and depression (r=-0.37, p<0.001 for both).

13. CHARACTERIZATION OF BONE DEFICITS IN PATIENTS WITH THALASSEMIA: A WINDOW OF OPPORTUNITY FOR INTERVENTION IN ADOLESCENTS

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Previous reports suggest that up to 60% of young patients with thalassemia (Thal) have low bone mass however, few studies have characterized these deficits. A better understanding of how Thal affects bone compartments (trabecular vs. cortical bone) and geometry is needed to develop optimal therapies. In this study, spine, hip and whole body aBMD were assessed by Dual Energy X-ray Absorptiometry (DXA, Discovery A, Hologic). Volumetric trabecular density (vBMD) and cortical content, thickness and strength (section modulus and strain strength index) were assessed in the distal tibia by peripheral Quantitative Computed Tomography (pQCT, XCT2000, Stratec). Z-scores were calculated using the Hologic pediatric database (DXA) and the Philadelphia Reference Data Project (pQCT). Fasting blood, pubertal status by validated self-assessment, dietary intake and physical activity by written questionnaires were collected. 40 subjects with Thal (20 Male, 17.6±5.2 y, Mean±SD) were compared to 34 ethnically matched healthy controls (15 M, 17.5±6.1 y). Subjects with Thal (27 β-Thal major, 7 E-β-Thal, 4 HbH and 2 Thal intermedia) had lower height and weight Z-scores

($p < 0.001$) and delayed age of menarche ($p = 0.009$) compared to controls. Bone formation markers were significantly reduced in young Thal (≤ 18 y) compared to controls, while resorption markers were elevated in adult Thal (both $p < 0.01$). BMD by DXA was significantly lower in Thal compared to controls at all sites assessed. Adult Thal had lower tibial trabecular vBMD ($p = 0.004$) and cortical BMC Z-scores ($p = 0.002$) compared to controls, and decreased cortical thickness ($p = 0.003$) and strength indices. Only cortical thickness was significantly lower in young Thal vs. controls ($p = 0.005$). In separate multivariate models, cortical thickness ($p = 0.029$) and spine BMD Z-scores ($p = 0.001$) remained lower in Thal vs. controls after adjustment for age, gender, puberty and body size. Physical activity was a significant predictor of hip Z-score ($p = 0.027$), and dairy intake predicted trabecular vBMD and bone strength after adjustment for age, gender, puberty and body size. In summary, bone deficits in Thal remain after adjustment for body size and puberty. Adult Thal subjects are characterized by lower trabecular BMD, smaller bone diameter and thinner cortical shell, leading to weaker bones at increased risk for fracture. Only cortical thickness and bone formation markers were compromised in young patients, suggesting a window of opportunity for nutritional and physical activity therapies during pubertal development.

14. HORMONE PARAMETERS AND BONE MICROARCHITECTURE IN β -THALASSEMIA MAJOR

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As a result of dramatically improved survival in thalassemia patients, previously not seen complications such as osteopenia and osteoporosis are recognized now in nearly 70% of these patients. Bone mineral density (BMD), measured by planar dual energy x-ray absorptiometry (DXA), is frequently used as a proxy measure of bone strength. Bone microarchitecture may be a better indicator for bone damage and fracture risk. In 22 β -thalassemia major patients (age: 13 - 43 y, 11 female), BMD of the lumbar spine and total hip was measured by DXA. In addition, we assessed the volumetric BMD and the bone microarchitecture of the non-dominant distal radius and distal tibia by high-resolution peripheral quantitative computed tomography (HR-pQCT), especially, the trabecular inhomogeneity parameter (TbSpSD), which characterizes the porosity of the spongiosa. Patients with thalassemia and diabetes mellitus ($n = 6$), hypothyroidism ($n = 8$) or growth percentile ≤ 3 ($n = 13$) did not reveal significantly higher radial TbSpSD values ($p = 0.21, 0.34$ and 0.30 , respectively). However, patients with hypogonadism ($n = 12$) had a porous or nearly dissolved spongiosa and differed significantly from non-hypogonadotropic patients with respect to radial TbSpSD (median 415 versus 177 μm ; $p = 0.04$), but not to DXA spine Z-score ($p = 0.4$). IGF-1 deficiency ($n = 15$) was also a significant predictor of higher radial TbSpSD values (median 469 versus 174 μm ; $p = 0.004$). Patients with anamnestic fractures ($n = 6$) had lower total densities ($p = 0.02$) and higher trabecular TbSpSD ($p = 0.02$) and initiated blood transfusions at an older age ($p = 0.023$), compared to those without fracture. However, DXA Z-scores did not reflect the fracture risk ($p = 0.1$) in this patient group. In these patients, the strongest predictors for a highly porous trabecular bone structure were hypogonadism and IGF-1 deficiency.

15. EFFECT OF TRANSFUSIONAL HEMOSIDEROSIS ON HEPATIC FIBROSIS IN EGYPTIAN β -THALASSEMIA MAJOR PATIENTS

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Regular blood transfusion puts β -thalassemia patients at a higher risk of developing hepatic iron overload and hepatitis C virus infection (HCV). Increased hepatic iron is assumed to potentiate progression towards liver fibrosis in chronic HCV infection. Our objective was to evaluate the potentiating effect of marked hepatic iron overload and chronic HCV infection on hepatic fibrosis in thalassemia patients. Sixty eight patients, previously diagnosed to have homozygous β -thalassemia and followed up at the Hematology Clinic of the New Children's Hospital of Cairo University (44 hepatitis C positive and 24 hepatitis C negative patients), were selected to participate in this study after signing a written informed consent. Their age ranged between 6 and 27 years with a mean age of 9.7 ± 2.1 years and compared to a group of 42 non thalassemic chronic HCV patients whose age ranged between 7 and 27 years with a mean age of 10.9 ± 1.5 years (control group). Liver Biopsies were done for all patients for estimation of stage of hepatic fibrosis and liver iron content (LIC). The results were then correlated to liver function tests and serum ferritin.

Our results showed that the stage of fibrosis and LIC were significantly higher in β -thalassemia patients than the non thalassemic HCV patients ($p = 0.005, p < 0.0001$ respectively). There was no significant difference between the two groups

of thalassemia as regards staging of fibrosis. LIC was significantly correlated to the degree of hepatic fibrosis in hepatitis negative thalassaemic group while ferritin was significantly correlated to the stage of hepatic fibrosis in thalassaemic patients with positive HCV.

In conclusion, hepatic iron overload had a potentiating effect on hepatic fibrogenesis in β -thalassaemia major while the hepatitis status did not affect the progress of fibrosis in these patients.

16. DO WE HAVE TO RECONSIDER FERRITIN OPTIMAL LEVELS IN MULTITRANSFUSED THALASSAEMIA MAJOR PATIENTS?

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Serum Ferritin levels are considered the gold standard for monitoring chelation treatment in iron overloaded Thalassaemia major patients (TMps). Over the time different values were proposed as the goal of optimal chelation. Nowadays, the introduction of T2* MRI have given new insight in iron deposition suggesting that even with low Ferritin levels a Tmp may develop complications.

50 transfusion-dependent TMps, mean age 30.8 \pm 2.03, switched from Desferrioxamine monotherapy to an intensive combined chelation with Desferrioxamine (40-60mg/kg/d) and Deferiprone (75-100mg/kg/d), on an individually tailored regimen. Ferritin was measured by immunoturbidimetric assay and calculated as the mean of monthly determinations in order to eliminate false positive results, since ferritin is an acute phase reactant. Cardiac and hepatic iron loading were investigated by Signa-MRI, 1.5-Tesla, T2*-sequences. LIC:liver iron concentration was calculated by Ferriscan. Cardiac function was assessed annually by echo-Doppler and endocrine function by dynamic tests (OGTT, GnRH) and hormonal screening. Statistical analyses were performed using SPSS (p<0.05 was considered significant).

After 5-7 years of combined chelation in 90% of compliant TMps, mean Ferritin decreased dramatically from 5.421 \pm 882ng/mL to 87 \pm 25ng/mL. A trend analysis, PROC MIXED in SAS, revealed a negative trend of Ferritin over time (p<0,0001) with a rate of decline=-65 ng/ml/month and a cumulative decrease. MRI measurements led to significant reduction (p<0.0001) of iron load to virtually iron free organs (T2*Heart from 13.6msec to 35.5msec & T2*Liver from 1.5msec to 33.2msec).

In 12/50 Tmp with pre-existing cardiac dysfunction on medication, symptoms reversed and heart medications were stopped. Ventricular dimensions and function in echo-Doppler shifted to normal. 9/14 Diabetic-2 (64%) and 17/19 (90%) with Impaired Glucose Tolerance normalized their glucose metabolism. The decrease in Glucose secretion (p<0.0001) and the increase in Insulin secretion (p<0.05) were correlated with a decrease in ferritin to normal levels and the normalization of MRI T2*L and LIC. 7/14 males with pre-existing Hypogonadism (50%) reversed and stopped testosterone injections. Correlations were established between the increase of testosterone and the decrease in ferritin & LIC to normal levels. No serious adverse events which led to permanent interruption of combined chelation were observed in our TMps even with ferritin levels <500ng/mL.

The use of combined chelation, by achieving a negative iron balance and a reduction of total body iron, induces the reversal of iron-load complications in the majority of TMps. This was correlated with the dramatic decrease of ferritin to normal levels. Additionally the fact that no serious adverse events have occurred might lead us to reconsider Ferritin optimal levels in TMps.

17. THE OH BOMBAY PHENOTYPE IN A CHILD WITH BETA THALASSEMIA MAJOR

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The male infant was diagnosed with beta thalassaemia major (homozygous IVS-1-5(G>C)) at five months of age after presenting with anaemia and failure to thrive. His parents were first cousins from India and carriers of beta-thalassaemia. His blood group at 5 months was O Rh positive with a negative antibody screen.

Pre-transfusion testing at seven months demonstrated a pan-reactive antibody (lacking specificity) against all group O cells tested and incompatible cross-match with group O packed cell units; the autoantibody screen and the direct

antiglobulin test were negative. The Oh Bombay phenotype was confirmed by the detection of anti-H antibody. The FUT1/FUT2 genotype confirmed "Classic Oh Bombay" with homozygous silencing of both.

The Oh Bombay blood group is rare. The Australia Red Cross Blood Service had at the time of diagnosis no Oh fresh units available, very few donors and 6 red cell units from homologous donations frozen in storage. Therefore, long-term monthly blood transfusions are not feasible in Australia and early haemopoietic stem cell transplantation (HSCT) is the most appropriate treatment for long-term survival. Prior to HSCT transfusion support has included Australian frozen Oh units as well as imported fresh units.

Rituximab (375mg/m²/dose) was given in an attempt to reduce anti-H titre in preparation for HSCT. This was effective in reducing anti-H to titres from 1:8 to 1:1, undetectable in the routine antibody screen. The patient is currently being conditioned with a Treosulphan/ Fludarabine/ Thiotepa and ATG for an unrelated mismatched (5/6) cord blood transplant from a group AB donor.

18. BABESIOSIS INFECTION IN TRANSFUSION DEPENDENT THALASSEMIA PATIENTS DURING THE PAST DECADE

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BACKGROUND: Between 2006 and 2008, three Thalassemia Major (TM) patients (pts) from the Thalassemia Center at the New York Presbyterian Hospital/Weill Cornell Medical College have been diagnosed and treated for babesiosis, an intraerythrocytic infection associated with fever, viral-like symptoms and hemolysis. Babesiosis is transmitted by the deer tick and has become problematic in the northeast owing to the increasing deer population.

METHOD: A chart review of 126 regularly transfused TM pts in our center was conducted and 3 pts (2F/1M, aged 23 - 40 years) were identified with babesiosis.

RESULTS: Three pts who were chronically transfused to maintain a pre-transfusion hemoglobin (Hgb) level of 9 - 10 gm/dl who had been splenectomized were identified with babesiosis. All had received leukocyte depleted washed red blood cells (RBCs). Each pt had a history of 1 - 4 weeks of low-grade intermittent fevers, fatigue and viral-like symptoms with increased hemolysis and Hgb levels dropping to 7 - 8 gm/dl. RBC smears revealed intraerythrocytic babesia confirmed with DNA PCR. Tracking of previous blood donors identified 2/3 pts received RBC units contaminated with babesia from asymptomatic donors. The third pt co-infected with Lyme had vacationed in a deer infested region of North Carolina. All pts were treated for babesiosis with azithromycin and atovaquone and symptomatically recovered with negative blood smears however, babesia DNA PCR remained persistently positive for 6 - 26 months.

CONCLUSION: Babesiosis should be considered in the differential diagnosis of a chronically transfused TM pt who presents with symptoms of a viral-like illness, persistent low-grade fevers and exaggerated hemolysis.

19. QUALITY OF LIFE OF THALASSEMIA PATIENT IN THE WEST BANK OF PALESTINE: REPORT OF A PILOT STUDY

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Thalassemia is common among Palestinians living in the occupied Palestinian territory. Although prevention efforts have been successful, there is limited research on the current health status of those patients living with thalassemia. A pilot study of the quality of life (QOL) of thalassemia patients from thalassemia patients living in the West Bank of Palestine using a validated Arabic translation of the SF36 was devised to begin to address this gap. We hypothesized that, given the existing social and economic conditions in Palestine, thalassemia patients would have lower QOL than patients in Western countries. With permission from the clinic ethics review board, 13 patients (9 female; average age 24.4) were recruited into the pilot study. Patients report notably poor scores on their physical component summary (PCS) and lower scores on all 4 physical health subscales. Mental Component Scores (MCS) are comparable to published Western studies. Palestinian patients only report lower scores on their vitality and mental health, but better scores on their social function and emotional role. These pilot data illuminate some important issues with the clinical care for thalassemia patients in Palestine. The comparable MCS scores suggest patient have a level of resiliency and ability to adapt to complex life situations. However, the lower PCS suggest there are challenges to the care for thalassemia patients living in a developing country experiencing war where there are shortages of clinical resources and access to inexpensive medication. In order to fully understand the situation of thalassemia patients in Palestine, a comprehensive study of the quality of life of thalassemia patients needs to be conducted.

20. ITHANET – THE COMMUNITY PORTAL FOR THE THALASSEMIAS AND OTHER HEMOGLOBINOPATHIES

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Online resources for the thalassemias and other hemoglobinopathies are largely limited to web sites of patient support groups and specialized databases. The migration of carriers and the severity, ubiquity and surprising genetic complexity of the thalassemias, however, call for an integrated web site to pool the expertise of all scientific and health professionals involved.

The ITHANET Portal (<http://www.ithanet.eu>) represents an expanding resource for clinicians and researchers dealing with hemoglobinopathies and a port of call for patients of hemoglobinopathies in search of professional advice. The ITHANET Portal now integrates a main page with forums and topical information on news and events, wiki-based content of protocols, clinical guidelines and related keywords and databases of thalassemia-related organizations and thalassemia mutations and frequencies.

The ITHANET Portal aims to become a comprehensive resource for all information relating to the hemoglobinopathies. As an interactive community tool it invites contributions to its content, including news and events items, research and diagnostic protocols, forum discussions, and contact information for clinical, research and diagnostic centers and patient organizations for hemoglobinopathies.

21. THE IMPACT OF THALASSEMIA ON SOUTHEAST ASIAN AND ASIAN INDIAN FAMILIES: A QUALITATIVE STUDY

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Few studies have examined the socio-cultural and socio-demographic issues faced by SE Asian and Asian Indian families affected by thalassemia, who comprise a growing segment of US thalassemia programs. Our objective was to learn about the challenges faced by these families to develop effective outreach strategies. We conducted 1-on-1, semi-structured interviews with 7 SE Asian (Lao, Hmong, Vietnamese, Cambodian) and 7 Asian Indian (Indian, Pakistani) parents of children (mean, 12.3 yrs) with transfusion-dependent thalassemia using a 13-question moderator's guide that addressed the impact of thalassemia on child and family, social support mechanisms, and general barriers to healthcare. All parents and 25% of their children were non-US born. Interpreter services were offered, but all parents preferred to be interviewed in English. Transcripts were analyzed using the constant comparative method and grounded theory. Parents cited frequently the emotional impact of thalassemia on themselves and their children. Transfusions and chelation therapy were especially difficult for their children. These perceptions were most often tied to parental hope for a cure. Although social support was available to them, few parents used resources beyond immediate family members due to perceived thalassemia-related knowledge gaps in the general public. Parental and physician knowledge gaps, financial constraints and complexity of disease management also emerged as occasional barriers to care. Our findings may provide a structural framework for developing a needs-driven set of initiatives aimed at improving the medical care and lives of this emerging population.

22. MRI T2* QUANTIFICATION OF CARDIAC IRON LOAD IN THALASSEMIA

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Measurement of cardiac iron presents a major challenge as neither serum ferritin, nor liver iron, are reliable indicators of cardiac iron overload. Quantifying cardiac iron by T2 star (T2*) provides useful insight into severity of myocardial siderosis. 60 regularly transfused Thalassemia patients ages ranging from 6 to 25 years (mean 17 yrs) and 10 healthy age-matched controls had cardiac iron quantified using a single slice multi echo T2* MR sequence (Siemens, 1.5 Tesla). Serum ferritin was measured and correlated with corresponding cardiac iron levels. Relation between cardiac iron T2* of patients receiving different modes of chelation therapy was studied. Those receiving combination of DFO+DFP had better

mean cardiac iron T2* (value of 27ms) compared to those on DFP therapy alone (22ms), although this difference was not statistically significant. Cardiac siderosis was noted to increase with advancing age and was seen in 50% cases (moderate i.e. 20-10ms in 20 , and severe i.e. <10ms in 10 patients.). There was no correlation between serum ferritin levels and cardiac T2* values . There was a statistically significant difference (p <0.01) in cardiac T2* between study population (mean = 23.45 ±13.4) as compared to controls (mean = 32.67 ±2.68). There was no significant difference in the functional cardiac parameters i.e. ejection fraction, end diastolic and end systolic volumes on T2* of controls as compared to study population.

23. POST SPLENECTOMY PRIAPISM IN β – THALASSEMIA INTERMEDIA – A CASE REPORT

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Introduction: Priapism, an unwanted painful erection that may last for more than 30 min as result of primary hematological disorder, has already been discussed both in sickle cell disease and sickle cell – β thalassemia. But as result in thalassemia intermedia, have been only 4 cases and we present an additional one.

Case report: A 45 year – old, male patient with β – thalassemia intermedia, has been followed in our Hospital for at least 7 years. He did not receive any transfusions for 10 years. In his past medical history, a Parvo B19 viral infection. Also he reported frequent intermitted use of sildenafil and ‘penile injections’ to treat an erection dysfunction. Due to increased splenic dimensions (23 x 15 x 8 cm) and hypersplenism (leucopenia, neutropenia and thrombocytopenia), the patient underwent a laparoscopic splenectomy – cholecystectomy. Before the splenectomy, he was assessed for thrombophilia with a border line value of ATIII and slightly reduced values of plasminogen and protein S. Following the splenectomy, while he was hospitalized, he was treated with LMW heparin and he changed to cumarin anticoagulant with therapeutic goal INR 2 – 2,5. After the 4th postoperative week, the patient reported ‘unwanted painful morning erections’ with a duration of 30 min to 3 hours and he was subsequently admitted to a Urology Clinic for further evaluation. In that period, the therapeutic values of INR were not achieved and the PLTs count was 450.000 – 550.000. After 25 days of hospitalization and repeated blood aphaeresis from the erectile area, he was discharged asymptomatic. The patient returned to cumarin anticoagulant therapy as well as anagrelide. The INR stabilized between 2,0 – 2,5, the PLTs counts 250.000 – 350.000, the hemoglobin 11,0 – 11,5 gr and the erythroblasts between 5 – 8 % .

Conclusion: The pathogenesis of post splenectomy priapism in β - thalassemia intermedia has non been adequately studied and is considered extremely rare. In all of the cases reported, priapism developed in the first 1 – 3 months postoperative. A possible explanation is that the slightly increased platelet count along with increased erythroblasts resulted in an increase in blood viscosity. In other cases, platelet count showed further increase up to 1.000.000. There is a possibility that prophylactic use of lower dose of ASA or Clopidogrel from the 4th post-operative day on might have reduced the overall number of priapism cases, in addition to the anticoagulant treatment. In our patient, the development of priapism, is most likely related to the increased blood viscosity due to thrombocytosis and erythroblast count increase. It seems that there is no definitive platelet count that should be treated with PLT aggregation inhibitors, even if for other reasons (Thrombophilia) there is concomitant anticoagulant therapy. The use of anagrelide lowered the platelet count and subsequently diminished relapse. We will continue the treatment with lower dose of anagrelide and the cumarin.

24. LIFESTYLE CHOICES IN ADULTS WITH THALASSEMIA IN THE THALASSEMIA CLINICAL RESEARCH NETWORK LONGITUDINAL COHORT

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Introduction: Individuals with thalassemia experience a wide variety of medical complications. Lifestyle choices such as smoking, ethanol consumption, or a sedentary lifestyle can have a negative impact on the health of thalassemia patients and worsen these complications. A baseline of lifestyle choices has not previously been established for adults with thalassemia. In this study we investigate the lifestyle choices (drinking alcoholic beverages, smoking cigarettes, activity

level, marital status) of individuals with thalassemia and the association of gender, ethnicity/race with lifestyle choices as well as the relationship of lifestyle choices with adherence to chelation therapy. **Patients and Methods:** A total of 237 adults with thalassemia (31.6±9.5 years, range 18-58 years, 87.3% regularly transfused, 54.8% female, 57.8% white, 39.2% Asian) enrolled in the Thalassemia Clinical Research Network (TCRN) Thalassemia Longitudinal Cohort (TLC) study in North America and London were included in this data analysis. **Results:** 57% of participants reported being single. 81% described their activity level as moderate or higher. 49% of participants reported smoking at some point in their life, 25% had smoked at least 100 cigarettes, and 15% had smoked in the last year. 30% of participants reported never drinking alcoholic beverages in the past year, and 25% reported drinking at least 5 drinks in a day. White and male patients were more likely to smoke, and Asian participants were more likely to be single. Smoking, drinking, and marital status did not predict patients' self-report of adherence to chelation, as measured by comparison of doses taken (participant recall) to those prescribed. **Conclusion:** Unhealthy lifestyle choices including smoking cigarettes and excessive alcohol consumption are common in this thalassemia population. In contrast, most subjects engaged in healthy exercise routines. Health care providers should screen lifestyle choices and educate patients accordingly, with particular emphasis on the potential exacerbation of thalassemia and iron-related complications.

25. VALIDATION OF PHENOTYPIC SCORING SYSTEM FOR THALASSEMIA INTERMEDIA

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Thalassemia intermedia is genetically heterogeneous with clinical severity in between thalassemia minor and thalassemia major. Prediction of the clinical severity is difficult. Phadke et al have described a phenotypic scoring system for thalassemia intermedia (TI) considering several parameters. We validated the scoring system in a cohort of TI patients in the Hematology clinic of Advanced Pediatric Centre. The parameters scored were age at presentation, severity of anemia, growth retardation, hemolytic facies, need for blood transfusions and splenectomy. A total of 136 patients were included in the study. The mean age at onset of symptoms was 4+ 2.3 years. Majority of the cases received one or more transfusions prior to referral with transfusion frequency >2/year in 46.3 % cases. The mean HbF at diagnosis was 61+ 23.6% (range 10.4-98.6%). Splenectomy had been done in 22.7% cases. According to scoring system by Phadke et al, 43.2 % of the TI patients had non-severe thalassemia and 56.8% had severe thalassemia. A significant difference was also found between severe and non-severe TI when presence of jaundice was analysed (p=0.04). We propose that presence or severity of jaundice and also pulmonary hypertension can also be included in the scoring system. This would enable identification of severe TI early so that effective interventions can be planned accordingly.

26. STANDARDIZING QUALITY OF LIFE ASSESSMENT IN THALASSEMIA USING THE SF-36

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Aim: The aim of this study is to compare SF36 data from the Thalassemia Longitudinal Cohort (TLC) study to US national norms and published literature on quality of life (QOL) outcomes in thalassemia. **Methods:** The Thalassemia Clinical Research Network (TCRN), an NIH sponsored network of 16 major thalassemia centers in North America and London, gathered SF36 version 2 data from 245 patients in the TLC. The SF-36 is the gold standard for measuring QOL outcome, but has only recently been used in thalassemia. We compare these data with US norms and published data on thalassemia. **Results:** As expected, our data show that QOL is diminished in thalassemia patients. When compared to US norms, TLC patients had statistically significant worse QOL on 5 of the 8 SF36v2 subscales (physical functioning, role-physical, general health, social functioning and role-emotional) and on both physical and mental summary scales (p<0.05). Compared to other published studies, the TLC population reports higher values on most scales, notably physical and emotional roles, and social function. These may reflect social differences between the report sites. **Conclusion:** These data show that the TLC SF36v2 data comports well with published studies and suggests that the SF36v2 should become a standard instrument for assessing QOL in thalassemia.

27. FRENCH REGISTRY FOR SURVEILLANCE OF BETA-THALASSEMIA

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Beta-thalassemia is a very rare disease in France. With the support of the National Plan for rare diseases, a registry for Thalassemia patients living in France was implemented in 2005.

On February 2009, the French register included 268 beta-Thalassemia Major (TM) and 110 beta-Thalassemia Intermedia (TI) patients. At the last follow-up, the median age was 20 years (0.5-56yrs). 182 patients underwent splenectomy at a median age of 9 years. Among the 64 patients with positive HCV-antibodies, 26 were HCV-RNA positive. Median serum ferritin level (available for 95% of patients) was of 1240 and 479 ng/l for TM and TI patients, respectively. 50 patients (33 females, 22 TM) became a parent. During the years 2005-2006, 63% of the patients receiving chelation treatment were treated with Deferoxamine whereas over the years 2007-2008, Deferasirox was prescribed to 71% of the patients. Among the 268 TM patients, 52 were transplanted (42 successfully). The frequencies of complications in the 216 TM patients not transplanted were rhythmic abnormalities 8%, cardiac failure 10%, hypothyroidism, 10% and diabetes 6%. Hypogonadism was found in 48% of patients aged more than 15 years. The frequency of complications increased with age but median serum ferritin levels did not. Cardiac MRI, introduced in 2005 in the patient's monitoring, was used in 40% of the TM patients aged more than 10 years, during the years 2007-2008. Establishment of a registry for a rare disease not only allows monitoring of indicators relative to mortality and morbidity but may also improve the patient's quality of care.

28. EVALUATION OF OSTEOPATHY IN THALASSEMIA BY BONE MINERAL DENSITOMETRY AND BIOCHEMICAL INDICES

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Thalassemic patients have an increased incidence of bone disease due to imbalance between bone resorption and formation. Our aim was to evaluate osteopathy in thalassemia, ascertain its incidence and address early diagnosis for its prevention and treatment. We evaluated bone density of 42 patients (10-25 years) by Dual Energy X-ray Absorptiometry (DEXA) by Lunar prodigy system (GE healthcare). Serum calcium, phosphorus and alkaline phosphatase were studied. Urine C-terminal cross-linked telopeptide of type 1 collagen (crosslaps) a marker of bone resorption was evaluated by ELISA. Serum 25-OH vitamin D and osteocalcin as bone formation markers were evaluated by RIA. Parathyroid hormone and IGF-1, major regulators of bone metabolism were studied by chemiluminescence and EIA respectively. Serum ferritin was taken as a marker of body iron overload and evaluated by chemiluminescence.

Our results showed, 50% had osteoporosis and 31% had osteopenia by Z-score on DEXA, while 16% had hypocalcemia and 79% had hyperphosphatemia. Urinary crosslaps were high in 55% indicating increased bone resorption. 36% had increased osteocalcin levels, 62% had low vitamin D levels and 38% had high parathyroid levels. IGF-1 was low in 52%. Ferritin levels were high in all (Mean: 5344ng/dl).

This study reveals that there is increased bone resorption probably due to hyperparathyroidism secondary to hypovitaminosis D. BMD by DEXA should be evaluated annually after 10 years and earlier if symptomatic for early diagnosis of osteopathy to prevent morbidity in these children.

29. GROWTH IMPAIRMENT IN PATIENTS WITH MILD HEMOGLOBIN E/BETA THALASSEMIA

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Growth retardation is a common complication of β -thalassemia major from several studies. Previously, we have shown that this consequence was also common in severe cases with Hemoglobin (Hb) E/ β -thalassemia, mainly compound heterozygosity of Hb E and β^0 thalassemia mutations. However, there was no clinical study available on the growth pattern of Hb E/ β^+ thalassemia patients who have been classified as having mild disease. To assess the effect of disease genotypes, chronological age, Hb and serum ferritin levels on the physical growth, we analyzed a longitudinal

data in 18 Hb E/ α^+ thalassemia patients (9 males and 9 females) from 257 HbE/ α^+ thalassemia cohort from our center. Their age ranges between 1 year to 17 years of age with mean age of 4 years and median time of follow up is 4.5 years. The diagnoses were confirmed by DNA analysis; 9 α^-28 /Hb E, 5 α^-CD19 /HbE and 4 $\alpha^-3.48del$ /HbE. Serial physical examination including weight, height, liver and splenic sizes were collected. The measurement of weight and height were calculated into Z-score according to the standard of Thai children (same age group). The average baseline Hb was 9.46 ± 1.19 g/dl and 66% of cases have never been transfused in their life. Four patients (22%) required occasional transfusion (1-2 times/yr) whereas two patients (11%) required regular transfusion. Most of the patients studied, even in non transfused patients, had growth retardation and their height were more affected than weight. At 5 years of age, 1/6 had normal growth (Z-score < -0.5), 3/6 had mild growth retardation (Z-score -0.5 to -1) whereas 2/6 had moderate growth retardation (Z-score -1 to -2). At 10 years of ages, 2/7 had normal growth, 1/7 had moderate growth retardation and 4/7 were severe growth retardation (Z-score > -2). After 10 years old, 2/8 had normal growth, 2/8 had mild growth retardation, 1/8 had moderate growth retardation, whereas 3/8 had severe growth retardation. Growth retardation can be detected as early as the first year of life but these abnormalities were more apparent after 6 to 8 year of age. Even the degree of anemia and transfusion requirement in Hb E/ α^+ thalassemia patients was significantly less than those of α^+ -thalassemia major and severe Hb E/ α^0 thalassemia. However, a significant proportion of patients with this milder form of thalassemia already developed growth retardation compared to normal children. This data will be important for future comprehensive genetic counseling and appropriate treatment required in this patient population.

30. CORRELATION BETWEEN ACUTE HEMOLYTIC CRISIS AND RBC PROPERTIES IN VARIOUS TYPES OF THALASSEMIAS

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Acute hemolytic crisis following febrile illness or inter-current infection is common in thalassemias. Although the disease severity may contribute to different levels of infectious susceptibility, and so hemolytic incidence, however the role of red blood cell's properties on susceptibility to hemolysis among different genotypes has not been investigated. Therefore we test the RBC osmotic containment and resistance to mechanical and/or environmental factors among different thalassemia syndromes. Red blood cells from 38 patients including; 4 deletional Hb H ($--SEA/\alpha^+3.7$), 13 Hb H/Hb CS or PS ($--SEA/\alpha^+T\alpha^+$), 9 mild and 12 moderate severity cases of Hb E/ α^+ thalassemia (all $\alpha^+41/42/\alpha^+E$) were tested and they were free from transfusion at least 3 months. We calculated % red cell hemolysis using optical density (OD) by a single channel spectrophotometer. In addition, acidified saline buffer with different pH; 7.0, 6.0, 5.5 and 5.0 with different saline concentration (0.45, 0.5, 0.55 and 0.6%NSS) were prepared and used to determine % hemolysis. Hb H/HbCS or PS is most susceptible to hemolysis in series of hypotonic solution at pH 7.3 followed by moderate and mild degree Hb E/ α^+ thalassemia and deletional Hb H ($--/\alpha^+$) with statistically significant differences among groups. Similar finding was also observed when the pH of two hypotonic saline concentration (0.45% and 0.5% NSS) was reduced sequentially. Interestingly, in Hb E/ α^+ thalassemias who had identical genotypes, their osmotic containment appeared to be correlated with their clinical severity; cases with more severe phenotype showed higher percentage of RBC hemolysis compared to their counterparts who have lesser severity. In our clinic, the incidence of acute hemolytic crisis is highest in non-deletional Hb H (Hb H/CS) followed by moderate and mild Hb E/ α^+ thalassemia and deletional Hb H in which compatible with our in vitro findings. In Hb H disease, this finding could be explained by different levels of excess α^+ globin tetramers which can precipitate to the RBC membrane causing oxidative damages. However, in HbE/ α^+ thalassemia, other genetic modifiers controlling RBC properties might play role on different level of hemolysis and determining their different clinical severity.

31. LIVER IRON BY MRI-R2* IN COMPARISON TO MRI-R2 AND BIOSUSCEPTOMETRY

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Non-invasive measurement of liver iron concentration (LIC) by biomagnetic liver susceptometry (BLS) is a method defined by physical laws. Meanwhile more than 10000 patients were measured with low-temperature SQUID biomagnetometer systems. Novel quantitative MRI methods have been introduced worldwide, but cross-comparisons between BLS and MRI sequences are missing. As an alternative method for our BLS reference method, a breathhold MRI-R2* sequence was employed in patients transferred to our center for cardiac R2* assessment.

Patients with transfusional siderosis (n=34, β -thalassemia major n=24) underwent a gradient recalled ECG gated multi-echo sequence. $R2^*$ is determined from a mono-exponential fit with constant signal level offset to the echo-time dependent signal amplitudes averaged over one liver slice. A non-linear relationship, $R2^* = R2_0 + a \cdot LIC^b$ ($r^2 = 0.96$), was found in the range between 100 and 8000 $\mu\text{g/g}$. Fit convergence for $R2^*$ at $LIC > 6000 \mu\text{g/g}$ was poor due to the available first echo-time of 1.3 ms. Of more concern is the insensitivity of $R2^*$ estimations at $LIC < 1700 \mu\text{g/g}$ (10 mg/g dry wgt), which was also confirmed in longitudinal measurements. A comparison with formerly measured $R2$ rates resulted in a conversion factor of 4.9 ± 0.3 for the approved MRI- $R2$ calibration by St. Pierre et al (2005) using freeze-dried liver biopsies ($r^2 = 0.78$). The insensitivity in the lower LIC may be caused by our multi-echo sequence, additional T1 effects, or a redistribution of hemosiderin and ferritin iron. In certain patients, where liver susceptometry is spoiled by local magnetic contaminations (dental braces) or by obesity, we can now measure liver iron with our alternative breathhold MRI- $R2^*$ method with known limitations.

32. THE CHILDREN'S HEALTH QUESTIONNAIRE (CHQ): A USEFUL TOOL TO ASSESS PEDIATRIC QUALITY OF LIFE AND FAMILY FUNCTION IN THALASSEMIA

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The aim of this study was to examine the utility of using a standardized Quality of Life (QOL) instrument to assess pediatric thalassemia patients and their families. 99 pediatric patients in the Thalassemia Longitudinal Cohort (TLC) study of the Thalassemia Clinical Research Network (TCRN) were studied using the Children's Health Questionnaire (CHQ), PF28. The PF28 is a 28 item, self-administered survey filled out by the parent. When data from the TLC is compared to the US norms, parents assessed their child's physical health significantly lower on their physical functioning and general health ($p < .0001$). Psychological measures shows parents assessed their child as having better self-esteem ($p = .0006$). Assessments of the family context showed that the child with thalassemia limited family activities ($p < .0001$), impacted parental time ($p = .005$) and emotions ($p < .0001$), but, even with these problems, families had better cohesion ($p = .02$). Overall, parent show a significantly lower assessment of their child's physical health ($p < .0001$) but no significant differences in psychosocial assessment ($p = .81$). The CHQ PF28 appears to be a useful tool for assessing the quality of life of children with thalassemia and how they impact the family.

33. PANCREATIC IRON LOADING PREDICTS CARDIAC IRON LOADING IN THALASSEMIA MAJOR

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Diabetes mellitus and cardiomyopathy are common in chronically-transfused thalassemia major (TM) patients, occurring in the second and third decades of life. We postulated that pancreatic iron deposition would precede cardiac iron loading, representing an environment favorable for extrahepatic iron deposition. To test this hypothesis, we examined pancreatic and cardiac iron in 131 TM patients over a 4 year period.

Cardiac iron ($R2^* > 50 \text{ Hz}$) was detected in 37.7% of patients and pancreatic iron ($R2^* > 28 \text{ Hz}$) in 80.4% of patients. Pancreatic and cardiac $R2^*$ were correlated ($r^2 = 0.52$), with significant pancreatic iron occurring nearly a decade earlier than cardiac iron. A pancreatic $R2^* < 100 \text{ Hz}$ was a powerful negative predictor of cardiac iron and pancreatic $R2^* > 100 \text{ Hz}$ had a positive predictive value of more than 60%. In serial analysis, changes in cardiac iron were correlated with changes in pancreatic iron ($r^2 = 0.33$, $p < 0.0001$), but not liver iron ($r^2 = 0.025$, $p = 0.25$). As a result, pancreatic $R2^*$ measurements offer important early recognition of physiologic conditions suitable for future cardiac iron deposition and complementary information to liver and cardiac iron during chelation therapy. Staging abdominal and cardiac MRI examinations could significantly reduce costs, magnet time, and need for sedation in young patients.