

HBOT + Stem Cells in Near Fatal Congenital Cardiomyopathy and some other Intractable Disorders

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It's Time



Open the window of
your mind.

Throw out the trash

Let in fresh air and
ideas

PLEASE REMEMBER



Extracts from Helsinki Protocol - 1

The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

The Declaration of Geneva of the WMA binds the physician with the words, “The health of my patient will be my first consideration.”

International Code of Medical Ethics declares that, “A physician shall act in the patient's best interest when providing medical care.”

Physicians must immediately stop a study when the risks are found to outweigh the potential benefits or when there is conclusive proof of positive and beneficial results.

PLEASE REMEMBER



Extracts from Helsinki Protocol- 2

In the treatment of a patient, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should be made the object of research, designed to evaluate its safety and efficacy.

In all cases, new information should be recorded and, where appropriate, made publicly available.

THIS IS THE PURPOSE OF THIS LECTURE

What is Carnitine Transporter

It's a tongue-twister.
Just remember
the first point.

Needed for transfer of long-chain fatty acids across the inner mitochondrial membrane for its utilisation/ oxidation.

Requires enzymes and transporters that accumulate carnitine within the cell (OCTN2: carnitine transporter), conjugate it with long chain fatty acids (CPT1: carnitine palmitoyl transferase 1), transfer the acylcarnitine across the inner plasma membrane (CACT: carnitine -acylcarnitine translocase), and conjugate the fatty acid back to Coenzyme A for subsequent beta oxidation (CPT2: carnitine palmitoyl transferase 2).

- Primary Carnitine Deficiency and Cardiomyopathy, Lijun Fu et al, <http://dx.doi.org/10.4070/kcj.2013.43.12.785>
- Disorders of carnitine transport and the carnitine cycle, Nicola Longo et al, Am J Med Genet C Semin Med Genet. 2006 May 15; 142C(2): 77–85

Deficiency result of Carnitine Transporter

Deficiency of the OCTN2 carnitine transporter causes primary carnitine deficiency, characterized by increased losses of carnitine in the urine & decreased carnitine accumulation in tissues.

Possible Presentations:

1. Skeletal and **CARDIAC MYOPATHY**
2. Hypoketotic hypoglycemia and hepatic encephalopathy

Management that usually but NOT always, works

Treatment for carnitine consists in a low-fat diet supplemented with medium chain triglycerides that can be metabolized by mitochondria independently from carnitine, (mega-dose) Carnitine supplements, & avoidance of fasting and sustained exercise.

- *Mitochondrial Cardiomyopathy, Deborah E. Meyers et al, Texas Heart Inst J. 2013; 40(4): 385–394*

Carnitine Transporter Deficiency Cardiomyopathy

Carnitine Transporter Deficiency induced cardiomyopathy is a rare autosomal recessive genetic disorder, characterized by plasma membrane carnitine transport defect, impairing uptake and oxidation of long chain fatty acids at the mitochondrial level

- *A. Cano et al, Ped. Cardiol. 2008; 29(1): 163-165*

This may cause oedema, sometimes therapy resistant heart failure, metabolic acidosis, hypoglycaemia, muscle weakness.

Investigations show normal PFT, ECG with giant T waves with prolonged QT intervals, subnormal blood carnitine levels and highly abnormal Echo Cardiogram

The case of QAB: severe carnitine transporter deficiency cardiomyopathy

QAB was diagnosed at Aga Khan University (AKU) Hospital, Karachi, as a progressive case of Congenital Cardiomyopathy in childhood due to of Carnitine Transporter Deficiency.

Family History: 2 pre-teen brothers earlier succumbed to this disease. One sister and one brother seem to have been spared. QAB affected. He had been on heart failure medications and mega dose L-carnitine as per prevailing medical practice, which had helped to prolong his life till 16 years with relapse and remissions. He exhibited slowly deteriorating heart failure, in spite of optimum recommended carnitine supplementation plus heart failure therapy.

Presented in Jan. 2015 with LVEF 20-25% as recorded at AKU, Fortis C-DOC Hospital & NHI, with breathlessness on climbing 2 flights of stairs.

LABOR FÜR STOFFWECHSELDIAGNOSTIK

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An
Att. Bushra Afroze, Paeds Dept
Fax: 0092214934294

-74800 Karachi/Pakistan

04.08.2008

Test for
Diagnosis

Name: [REDACTED]
Probeneingang: 21.07.08

Geb.Dat.: 27.04.00
Probennr.: 6549

Acylkarnitinprofil und Karnitinstatus

Referenz

Gesamtkarnitin:	*2	µmol/l	7 - 70
Freies Karnitin:	*1	µmol/l	5 - 45

Acylkarnitinprofil/Interpretation:

No measureable free carnitine or acylcarnitines. Carnitin transporter defect ?

Prof. Dr. O. Bodamer

DI. Dr. A. Mühl

Pulmonary Function Test

Name : ██████████ M.R. No. : 114-18-49 Age : 14 Y
 Rpt. No. : 2014:PFT03312 Date : 17/12/2014 12:41 Sex : Male
 Ref. Dr. : MEHNAZ ATIQ AHMED Source : CC-PAED

Height (cm) : 157 Weight (Kg) : 39 BMI (kg /m²) : 15.60

Cough : N Chest pain : N Sputum : N Dyspnoea : N Koch's : N Wheezing: N Allergy : N
 Smoker : N Ex-Smoker : N Duration : Smoking Quit Date : Cigarette Per Day :

Medication : None.

Clinical History : Pre-op.

Reason For Test : None.

Pulmonary Parameters											
Pre-Bronch				Post-Bronch				Lung Volumes			
<i>Spirometry</i>				<i>Spirometry</i>							
	Actual	Pred	% Pred		Actual	% Pred	% Chng	Actual	Pred	% Pred	
FVC	2.33	3.25	71	FVC	2.41	74.00	3	TLC		---	
FEV1	1.97	2.78	70	FEV1	1.96	70.00	0	IC	1.25	2.52	- 49
FEV1/FVC %	84.00	86.00	98	FEV1/FVC %	81.00	94.00	-3	ERV	1.10	0.73	150
FEF (25 - 75 h)	2.06	3.16	65	FEF (25 - 75 h)	2.04	64.00	0	RV			-
Exp. Time (sec)	6.77			Exp. Time (sec)	6.89		1				
SVC.	2.35	3.25	72	SVC.							

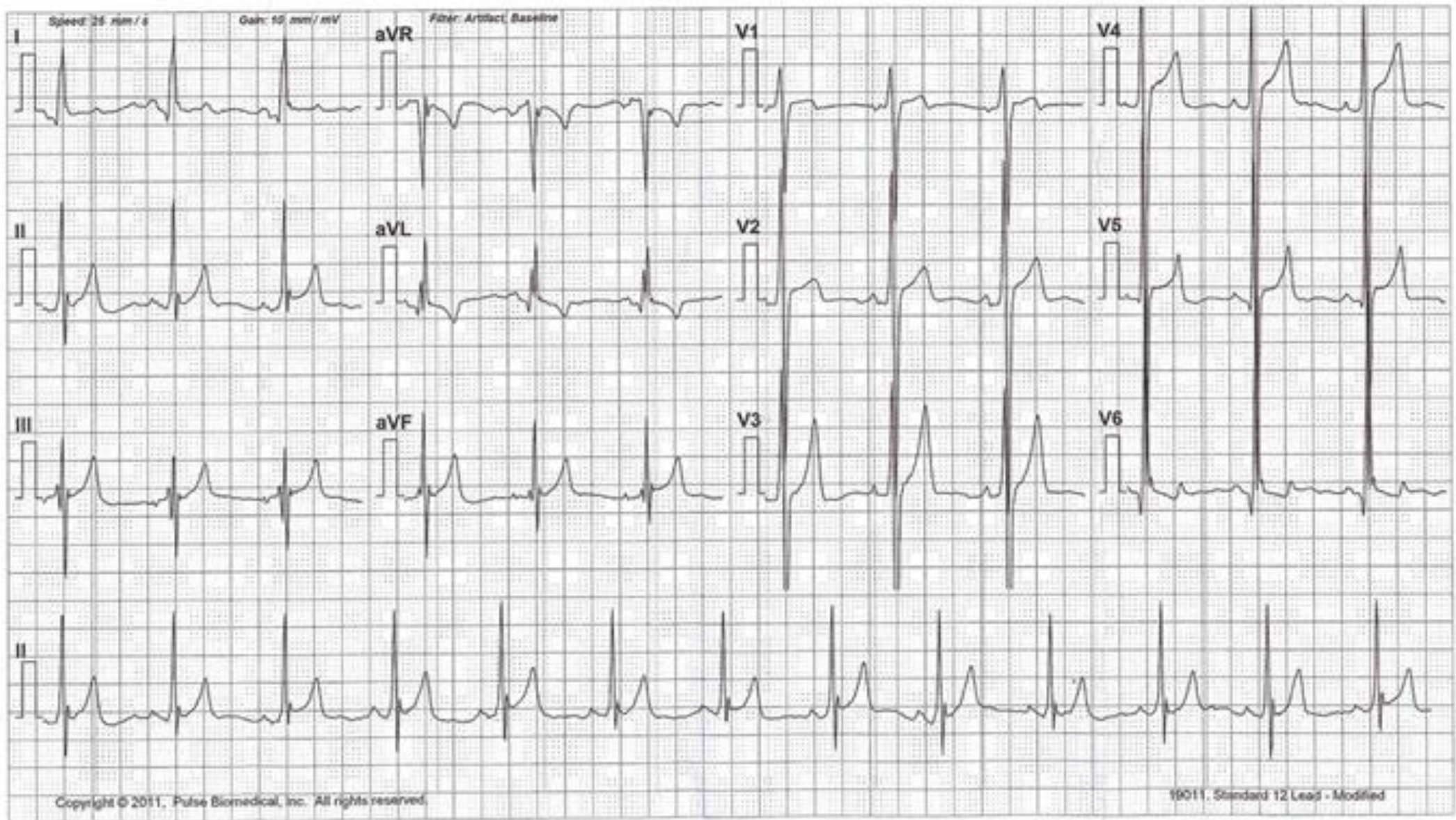
Conclusion

Non Specific impairment by spirometry.

No Significant improvement after bronchodilators.

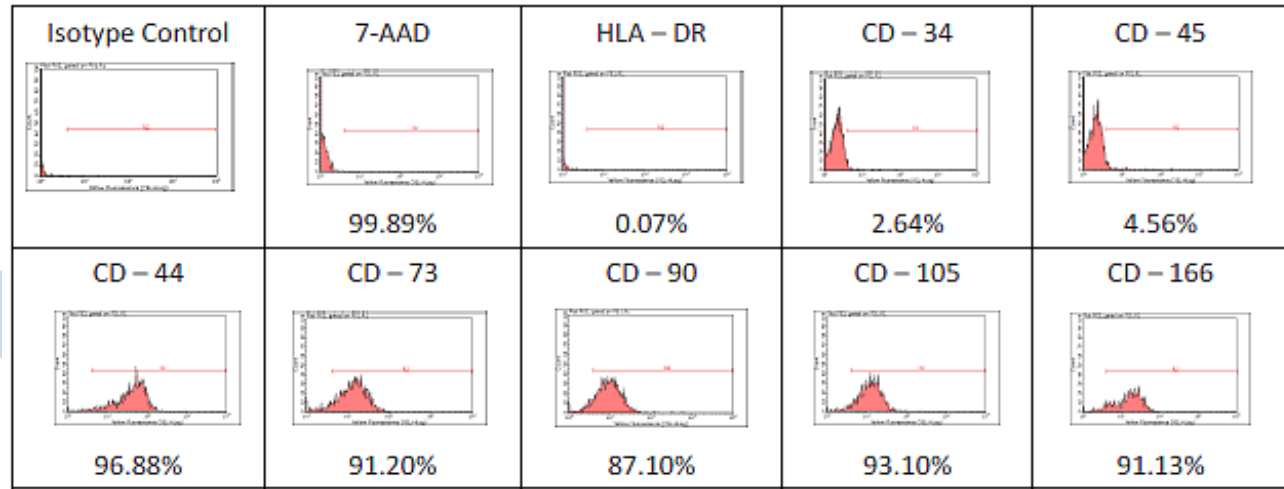
Lung volumes with DLCO is recommended for further clarification.

EKG



Major section of COA of MSC used

Immunophenotyping



Test Results:

TEST	SPECIFICATION	RESULT
Viability (dye exclusion or flow Cytometry)	> 85%	99.89%
Endotoxin (LAL Assay)	< 0.06 EU/mL	< 0.06 EU/mL
Sterility	Negative	Negative
Mycoplasma (PCR Assay)	Not Detected	Not Detected
Immunophenotyping (Flow Cytometry)	HLA - DR < 5%	0.07%
	CD - 34 < 5%	2.64%
	CD - 45 < 5%	4.56%
	CD - 44 > 80%	96.88%
	CD - 73 > 80%	91.20%
	CD - 90 > 80%	87.10%
	CD - 105 > 80%	93.10%
CD - 166 > 80%	91.13%	
Karyotyping	Normal	Normal

Treatment given to QAB by us

Mega dose L-Carnitine + CHF therapies continued as at AKU Hosp. (Dr. M Atiq) plus salt restricted non-strainous life style.

HBOT 1.5 ATA with 100% Oxygen, 15 minutes to dive plus 15 minutes to surface plus 60 minutes at full pressure x 40 sessions. After 20 sessions, we proceeded to transplant Stem Cell.

- **OUR HYPOTHESIS:** The heart is merely starving and is showing secondary effects of this. Carnitine Transporters are proteins, majority synthesized in the LIVER. Why not help the liver also?
- Hence, we gave 1/3rd dose of fresh MSC calculated/kg body weight, in Coronary arteries to help the weakened heart & the rest 2/3rd in the liver to enhance liver Carnitine Transporter manufacture.

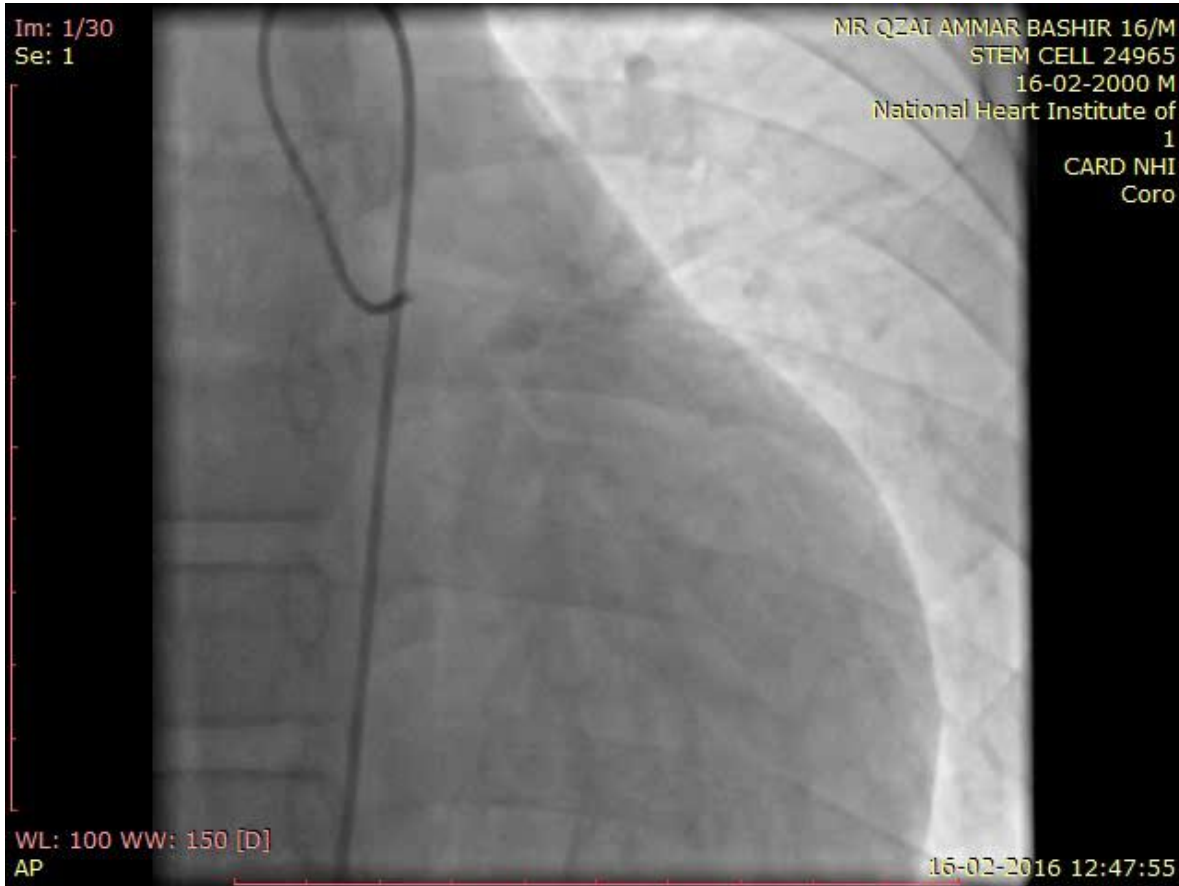
Serial LVEF Data

Table 1: LVEF data from Echo-Cardiogram record.

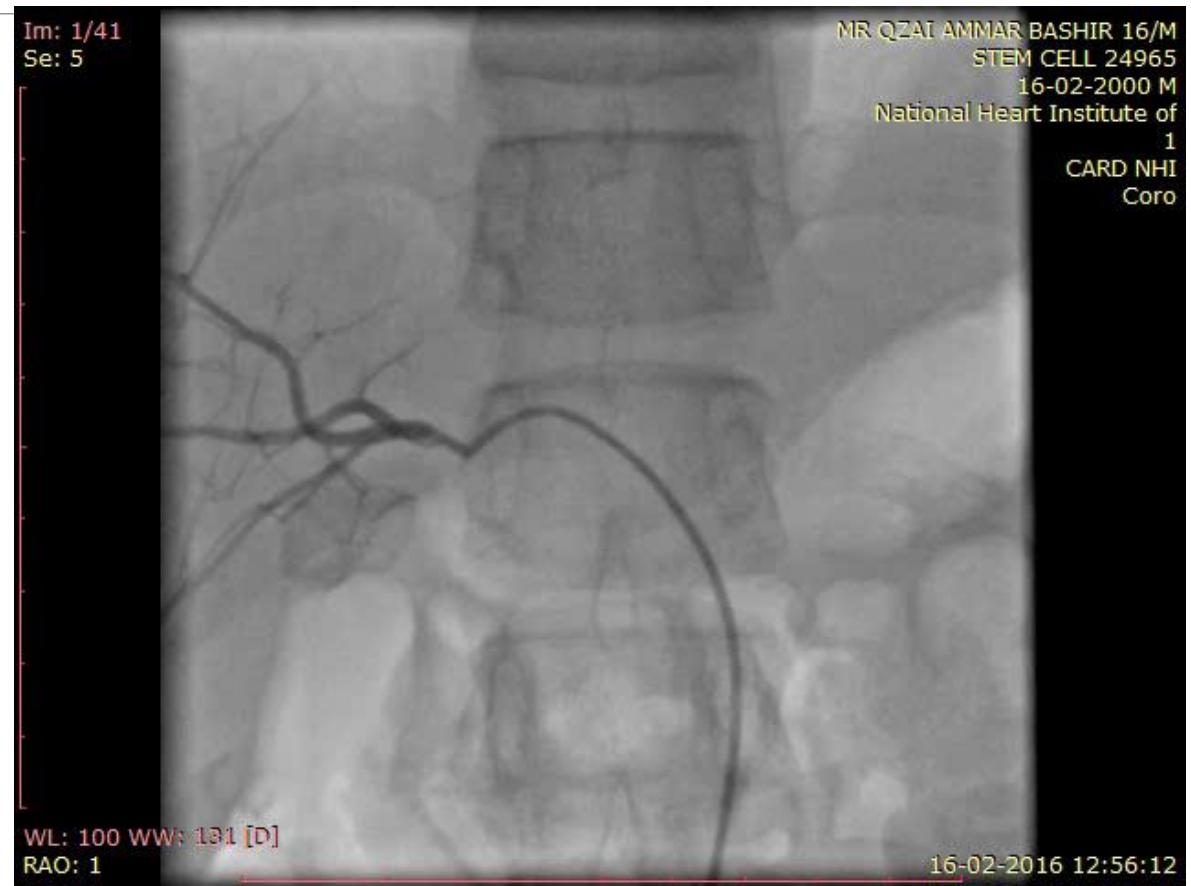
Date: Y/M/D	Where Done	LVEF %
2006/01/13	AK Univ. Hosp. Karachi	30
2006/12/29	AK Univ. Hosp. Karachi	59
2007/02/26	AK Univ. Hosp. Karachi	60
2007/06/04	AK Univ. Hosp. Karachi	60
2008/01/14	AK Univ. Hosp. Karachi	58
2008/05/05	AK Univ. Hosp. Karachi	34
2008/08/04	AK Univ. Hosp. Karachi	47 Dx done
2009/05/20	AK Univ. Hosp. Karachi	50
2009/10/14	AK Univ. Hosp. Karachi	54
2010/05/26	AK Univ. Hosp. Karachi	55
2010/12/31	AK Univ. Hosp. Karachi	70
2011/02/09	AK Univ. Hosp. Karachi	56
2011/08/22	AK Univ. Hosp. Karachi	50
2012/02/20	AK Univ. Hosp. Karachi	55
2013/01/02	AK Univ. Hosp. Karachi	30
2013/04/03	AK Univ. Hosp. Karachi	32
2013/04/15	AK Univ. Hosp. Karachi	26
2014/10/22	AK Univ. Hosp. Karachi	30
2015, January	Initial at Fortis C-DOC Hospital, Delhi	20 1 st Procedure
2015/02/31	After HBOT+SCT, Natl.Heart Inst,Delhi	35 Post-Proc.
2015 July	AK Univ. Hosp. Karachi	55
2016 January	Post Procedure, NHI, Delhi	55 2 nd Procedure
2017 January	AK Univ. Hosp. Karachi	84 Stable
2018, January	AK Univ. Hosp. Karachi	55 Neglecting Rx still LVEF Ok

Stem Cell delivery to QA

Heart & Liver as shown



HEART



LIVER

Outcome of therapy (Jan 2015 →)

LVEF went up from 20% to 35% (Fortis C-Doc Hospital, Delhi: immediate HBOT action) within 6 weeks, by February 2015

LVEF went up to 55% (delayed SCT Effect) by Mid 2015.

January 2016, LVEF maintained at 55%. (Normal)

1st Booster dose of HBOT + SCT given as before.

January 2017, LVEF reached 80 – 85% (Maximum normal)

Boy felt so good, he became like any naughty boy of his age, indulged in by his father who had already lost two sons to the disease.

He neglected regular medications, diet control & life style guidelines to a fair degree.

January 2018, LVEF still at 55%, doing well and enjoying normal life.

Discussion 1

This being a treatment failure case of carnitine transporter deficiency induced cardiomyopathy, who appeared to be in the last lap of his lifespan, we decided to use desperate measures under the provisions of Helinki Protocol Para 35.

Carnitine is a naturally occurring hydrophilic amino acid derivative, produced endogenously in the kidneys and liver and derived from meat and dairy products in the diet.

Cannot easily enter mitochondria and get oxidized for energy production and formation of ketones for brain function.

- *Fernando Scaglia (2014) Carnitine Deficiency*

Carnitine plays an essential role in the transfer of long chain fatty acids into the mitochondria for beta-oxidation.

- *Longa N, Amat C, Pasquali M (2003) The OVTN2 carnitine transporter and fatty acid oxidation. Membrane transporter disease, pp. 161-174.*

Discussion 2

We chose to use HBOT, prior to use of stem cell therapy based on the work of Diaz-Barbosa et al. in early 2000s.

They showed that stem cell therapy in the middle of HBOT, gave a greater benefit than either therapy alone.

- *JL Diaz-Barbosa & FJ Morales; (July 2003) 3rd Int. Symposium on Cerebral Palsy and the Brain Injured Child. Fort Lauderdale, Florida.*
- *JJ Catherine, Tonseth KA, Utheim TP (2017) Cultured epidermal stem cell in regenerative medicine. Stem Cell Res Ther 8(15): 155.*

We followed similar protocol with on-site transplantation

Conclusion:

Carnitine Transporter Deficiency Cardiomyopathy

No adverse reaction from our treatment protocol, even after 3 years.

It is difficult to quantify the relative degree of help obtained by this previously non-responder case, from HBOT, stem cell therapy and both given together.

Not enough cases per center to do a DBPCR Trial. Hence, going by this single safe and effective case study record, we propose to follow the same protocol for future cases if any, till we have more data.

We are also open to an international collaborative study

REGENERATIVE THERAPIES

**A NEW APPROACH IN MEDICINE
using HBOT + Stem Cells.**

**We have many more cards up our
sleeve, but that is for the next time**

Our Philosophy in NDD is to use:

LIFE STYLE MANAGEMENT

Multimode approach regulated by INDIVIDUAL NEED only.

- Comprehensive individual need based Standard Therapies: COMPULSARY
- Biomedical need-based approach, using special diets + micronutrients, with regular follow up adjustments as per age, sex, weight, need, etc. + drugs:

IF NEEDED

- HBOT, based on SPECT 3D Fusion Scan: IF NEEDED
- rTMS with NFB , based on SPECT 3D Fusion Scan: IF NEEDED
- Sensory Integration Therapy, based on Clinical Findings: IF NEEDED
- Ayurveda / Unani based brain repairing agents and antivirals: IF NEEDED
- VERY SELECTIVE cases for Stem Cell Therapy when no approved available therapy is giving desired results after 4 to 5 years age, done as per Helsinki Protocol

Special Education, ABA, Speech Therapy



Cognition, behaviour, memory, speech, oro-motor skills, etc. are mainly controlled by Prefrontal lobe, Basal Ganglia, Anterior Cerebellum, Thalamus, lower Parietal lobe

These therapies polish up the remnant neuronal functions in the inflamed hypoxic brain to bring out the best possible improvement in a child.

However, these therapies cannot resolve / repair / regenerate the damaged brain areas (Penumbra) involved in Inflammatory HIE.

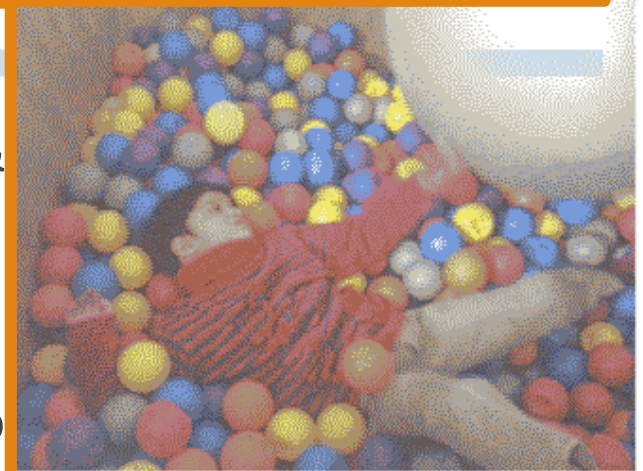
HBOT +/- SCT may, to a good extent



OT/PT, Sensory Integration Therapy



Hyper-sensitivity to sensations like sound, light, touch, hyperactivity, high selective muscle tone, aggressive behavior, cognition & behavior are controlled by Frontal and Prefrontal lobe, Cerebellum and basal Ganglia.



Physical Therapies including S/I may polish up the remnant neuronal functions in the remaining normal areas in these areas of brain to bring out the best possible improvement in the child.



However, they cannot regenerate the inflamed HIE lesions of damaged brain for augmented benefits.

HBOT +/- SCT may, to a good extent



Sensory Integration Laboratory augments effects of OT



Sensory Integration Therapy deserves special mention. It work to an extent we had not expected. The colour-changing glass fibre strands, the softly vibrating mattress, moving laser light show, bubble columns and soothing music helps calm down the child to enhance, concentration & reduce Attention Deficit Hyperactivity Disorder.

HBOT helps augment ALL these functions by healing the brain to a good extent.

Arts & Crafts and Group Activities

These group activities enhance socialization, eye-hand and mental concentration, learning to share and enjoy together

These are some of our basic instincts and abilities, mostly fine-tuned by the basal ganglia, cerebellum, temporal lobe, and practically all sensory motor and emotional areas.

Inflammatory HIE in these areas reduces above skills.

However, they cannot regenerate the inflamed HIE lesions of damaged brain for augmented benefits.

HBOT +/- SCT may, to a good extent



Extramural Activities to train them to live and perform in open society

The UDAAN Half-Marathon 2nd April 2017 on Autism Day



Planning Trustees
Marathon success.



National Spokesperson inaugurating the run
Our Team



Ambulance on standby
Felicitating the



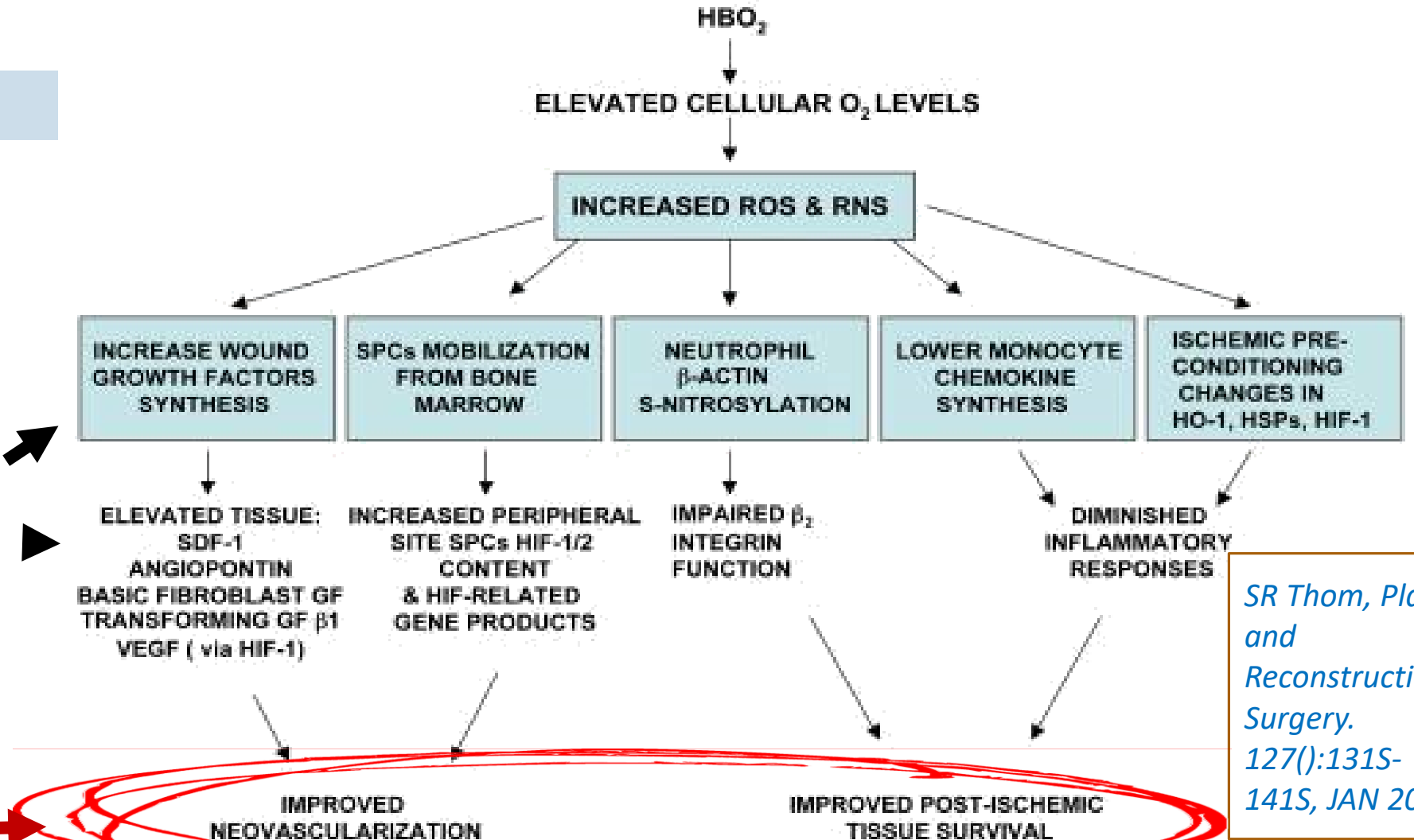
Felicitating Para-Olympics
Gold Medal Winners

How HBOT works: From high O₂ to Cytokine



You don't need to know why the Laptop functions.

But you must know what it can do.



SR Thom, Plastic and Reconstructive Surgery. 127():131S-141S, JAN 2011

Defining Stem cells (Incl. Mesenchymal)

Called Master Cells of the body.

- Undifferentiated cells. Have the ability to divide and differentiate into other 200 cell types.

Stem cells distinguished from other cell types by 2 important characters

- Stem cells are unspecialized cells capable of renewing themselves through cell division, sometimes after long periods of inactivity.
- Under certain physiologic or experimental conditions, they can be induced to become tissue or organ specific cells with special functions, like cartilage, liver tissue, heart muscle, pancreatic tissue, nerve tissue, blood cells etc.

Mesenchymal cells are HLA antigen-deficient stem cells

- <https://stemcells.nih.gov/info/basics/1.htm> (last accessed 1st May 2018)
- <https://medlineplus.gov/stemcells.html> (last accessed 1st May 2018)
- *Human mesenchymal stem cells - current trends and future prospective; Umrao Ullah et al, Biosci Rep. 2015; 35(2): e00191*
- *A Concise Review on the Use of Mesenchymal Stem Cells in Cell Sheet-Based Tissue Engineering with Special Emphasis on Bone Tissue Regeneration; Stem Cells International, Volume 2017, Article ID 2374161, 13 pages*

Dawn of a new era, using rHBOT / mHBAT with or without stem cells



Peak effect:
HBOT
4-6 mth
SCT
6-24 mth



*And many others
whereas I am
lucky to be
plucking the fruits*



- Refer also to:
Anti-inflammatory and immunomodulatory mechanisms of mesenchymal stem cell transplantation in experimental traumatic brain injury, Run Zhang et al; Journal of Neuroinflammation; 2013: 10:871

Totipotent (embryonic) Stem cells



CAN POTENTIALLY MAKE ANY PART, maybe even clone.

Generally not permitted due to ethical, moral, legal restrictions.

Small risk of cancer / tumor formation after 4 to 5 years

LONG TERM safety unknown though potency very good.

GVHD (Graft versus host disease) rarely seen but what happens at micro level is unknown;

Small stock.

Needs Culturing and often Cell Manipulation to obtain therapeutic dose of desired specific stem cell line.

- In culture, after about 3 passages, senescence or Genetic Micro-Array changes (mutation) may occur.
- Donor's tissue antigen start to appear
- Their long term effect not fully studied and published.

WE LIKE TO STAY AWAY FROM IT.

Bone Marrow Stem cell (BMSC)

Bone marrow derived mononuclear cells (high % of CD34+ but low Mesenchymal Stem cells) with no chemical added, has stood test of time (> 5 decades) as reasonably effective & safe with virtually no risk of tumor formation.

Though adequate stock available, there remains a problem with senescence of the stem cells inside the recipient body, hence efficacy less if donor is aged > than 12 years.

Antigen matching needed if allogenic donor blood used.

No infection, no allergy, no GVHD, if used in same patient.

BMSC Protocol for Stem Cell therapy

Requirement: > 2 - 4 million viable stem cells/ kg body wt, or more as per severity & # of sites

Practical: one site on Bone marrow prick yields 1.2 - 2 ml Marrow, rest is oozing blood: **useless**.

- Usually, using many pricks and twisting the needle tip, we get 60 ml of marrow

Donor age > 10 years, may have stem cells that do not last & multiply long enough in body to be optimally useful.

- Influences of age-related changes in mesenchymal stem cells on macrophages during in-vitro culture; Yuan Yin et al; Stem Cell Research & Therapy (2017) **8:153**

Culture (passages) up to 2 yield true antigen-less copies. After 3 to 4 passages, donor HLA antigens start to appear. NOW, tissue matching recommended as we need a lot of stem cells/patient, even in a child.

Storage: kept at -180C & transported at -80C, still 10 - 40% will degenerate. Overages needed

Peripheral Blood Stem cells

Good for autologous (self-) use for treating acquired lesions.

Peripheral Blood has Stem Cells but the pool is very small. Collected from blood by apheresis (as we sometimes do for platelets).

The blood stem cell pool may be temporarily enhanced by injection of specific chemicals to make the marrow release more stem cells.

CAUTION FOR ALLOGENIC USE: Micro-array genetic changes after 3 passages on culture not yet studied. Most cells will be CD34+ type.

If collected at age > 12 years, the problem of senescence comes in since these cells do not multiply long enough inside the recipient body.

Long term safety and potency is being studied by other researchers.

Cord Blood Stem Cells

Autologous

If used in same patient, very safe and quite effective. BUT, small stock. Must be cultured to get optimum quantity.

Long term viability issues after years of storage, even at -181C.

Cannot be used in genetic diseases in same patient as his SCs carry same defect.

Donor Pool

Pooled cord blood generally easily available.

- Tissue match recommended for safety.
- Proper handling, maintenance of sterility, donor history, infectious disease profile, etc. to be ensure stringently.
- Antigen matching needed if allogenic donor blood used.
- No infection, no allergy, no GVHD, if used in same patient.

UCBSC sources must comply with AABB, etc.

Umbilical Cord Tissue stem cells (UCTSC) Wharton's Jelly, Somewhat similar to Prochymal®

The umbilical cord and placenta contains a filler tissue called Wharton's Jelly comprising mostly the primitive Mesenchymal Stem Cells and a negligible population of hemopoietic Stem Cells (CD34+). Together, they offer a wider range of applications than either alone.

Relatively easily available after proper check-up of viability, infection profile, and standardization HLA etc. analysis).

Donated UCTSC appears useful in genetic diseases

UCTSC sources must comply with AABB, etc.

Minimal Criteria for MSC



Adherent to plastic

Surface antigens present:

- More than 95 % expression of CD-105, CD-73 & CD-90
- MSCs must NOT express (less than 2%) CD-14, CD-11b, CD-79a, CD-19, CD-34, CD-45 and HLA-DR)
- Must be capable of differentiating into various tissue types
- Naked cells that, till two passages of in-vitro culture, do not acquire the donor antigen. If obtained from umbilical cord Wharton's Jelly, enough material obtained to avoid more than 2 passages.

Int. Society for Cellular Therapy, Cytotherapy, 2006, Vol.8, No.4, Pg 3-15-317)

Mesenchymal Stem Cells (MSC)



Specific stem cells that closely resembles Embryonic Stem Cells

- These MSCs undergo Cell Fusion with injured resident Stem Cells.
- Resident stem cells in coordination with infused MSC helps to develop new cell types and repair injured ones.
- Needed to repair / replace a tissue type; e.g.:
 - Brain / nerve tissue in brain injury / inflammation
 - Heart muscle tissue in AMI
 - Pancreatic b cells in Diabetes
 - Liver tissue in Cirrhosis; can overcome insulin resistance
 - Lung tissue in COPD
 - Cartilage tissue in Osteoarthritis
 - Repair/Regeneration in genetic diseases like Thalassemia, DMD, Carnitine Transporter Deficiency CHD



Benefits of MSC

Promising therapeutic effects even though the transfused cells were, if at all, only barely detectable in the injured organs.

Secretes factors (Interleukins and Cytokines) that actively modulate debilitating local inflammatory reactions.

Reduces apoptosis, and fibrotic tissue remodeling

Recruits resident regenerative cells to contribute towards the beneficial effects

- *A. I. Caplan and J. E. Dennis 2006; E. M. Horwitz and W. R. Prather 2003.*
- *Treatment of stroke significantly reduces apoptosis within the peri-lesioned area, (J. Chen et al 2003).*

Redox Regulation by Stem Cells & HBOT

The low level of H₂O₂ in quiescent hematopoietic stem cells (HSCs) contributes to maintain their stem-ness, whereas a higher level of H₂O₂ within HSCs or their niche promotes differentiation, proliferation, migration, and survival of HSCs or stem/progenitor cells.

Excess amounts of ROS create an inflammatory and oxidative micro-environment → cell damage and apoptosis of stem and progenitor cells.

HBOT promotes progenitor cell expansion and mobilization from BM, leading to reparative neovascularization and tissue repair in pathophysiological states such as aging, atherosclerosis, heart failure, hypertension and diabetes

Understanding these molecular mechanisms will lead to the development of novel therapeutic strategies.

- *Adapted from Redox regulation of stem/progenitor cells and bone marrow niche; Norifumi Urao, Masuko Ushio-Fukai, Dept of Pharma, Center for Lung and Vascular Biology, Center for Cardiovascular Research; Univ. Illinois, Chicago; Free Radic Biol Med. 2013 January ; 54: 26–39*

S.Cs regulate Cytokines just like HBOT

Stem cells down-regulate pro-inflammatory Cytokine after injury and reverse the cytokine effect in 2 to 3 days of injury

- Anti-inflammatory and immunomodulatory mechanisms of mesenchymal stem cell transplantation in experimental traumatic brain & immunomodulatory mechanisms of mesenchymal stem cell transplantation in experimental traumatic brain injury;
- *Run Zhang et al; J. of Neuro-inflammation; 2013: 10: 871*

Mobilisation of BM Stem Cells

HBOT mobilizes bone marrow derived-stem/progenitor cells by a free radical mediated mechanism

Post-treatment values of CD34+, CD45-dim leukocytes were always 2-fold greater than the pre-treatment values.

The authors concluded that putative progenitor cell mobilization is higher with HBOT, and all newly mobilized cells exhibit higher concentrations of an array of regulatory proteins.

- *CD34+/CD45-dim stem cell mobilization by hyperbaric oxygen – changes with oxygen dosage; SR Thom et al; Stem Cell Res. 2014 May; 12(3): 638–645*
- *Efficient Homing of Multipotent Adult Mesenchymal Stem Cells Depends on FROUNT-Mediated Clustering of CCR2; Fikru Belema-Bedada et al: Cell Stem Cell, Volume 2, Issue 6, 5 June 2008, Pages 566-575,*

Composition of Adult Stem cells

CD 34+ cells are hemopoietic stem cells. A primitive subset - CD133+ cells differentiate into neurons.

Mesenchymal stem cells are Primitive Cell progenitors (comparable to embryonic stem cells). They can release Glial derived neurotrophic factor (**GDNF**). GDNF can rescue neurons from a lack of oxygen and help stimulate the repair of white matter in the brain.

CD34+ stem cells can also release **Neurotrophin 3 (NT-3), Nerve Growth Factor (NGF) and Brain Derived Neurotrophic Factor (BDNF)**, which can stimulate the growth of new neurons. They help balance the immune system and autoimmune disorders, relax the artery walls which promotes improved blood flow. Cerebral palsy or TBI is characterized by white matter damage and is the best responder to adult stem cell treatments.

Biological effects of Stem Cells

Adult MSCs are capable of dividing into one of several mesenchymal phenotypes such as osteoblasts, chondrocytes, myocytes, marrow stromal cells, tendon-ligament fibroblasts, and adipocytes.

These MSCs secrete a variety of cytokines and growth factors that have both paracrine and autocrine activities. They have bioactive factors suppress the local immune system, inhibit fibrosis (scar formation) and apoptosis, enhance angiogenesis, and stimulate mitosis and differentiation of tissue-intrinsic reparative or stem cells. These effects, which are referred to as trophic effects, are distinct from the direct differentiation of MSCs into repair tissue. Several studies which tested the use of MSCs in models of infarct (injured heart), stroke (brain), or meniscus regeneration models examples of MSC-mediated trophic effects in tissue repair.

- *Mesenchymal stem cells as trophic mediators; A. I. Caplan et al, J. Cell. Biochem. 98: 1076-1084, 2006*

Spastic paresis after perinatal brain damage in rats is reduced by human cord blood mononuclear cells

Therapeutic potential examined for human umbilical cord blood derived mononuclear cells containing multipotent stem cells to facilitate motor recovery after cerebral hypoxic-ischemic damage in neonatal rats.

- Left carotid artery ligation followed by resuscitation was done.
- Histologic and immuno-histochemical analysis on postnatal d 21 revealed that neonates developed severe cerebral damage after the hypoxic-ischemic insult.

After transplantation of human umbilical cord blood derived mononuclear cells, spastic paresis was largely alleviated, resulting in a normal walking behaviour.

- This effect was accompanied by the fact that mononuclear cells had entered the brain and were incorporated around the lesion without obvious signs of trans differentiation.

Their study demonstrated that intraperitoneal transplantation of human umbilical cord blood derived mononuclear cells in a rat model of perinatal brain damage leads to both incorporation of these cells in the lesioned brain area and to an alleviation of the neurologic effects of cerebral palsy as assessed by footprint and walking pattern analysis.

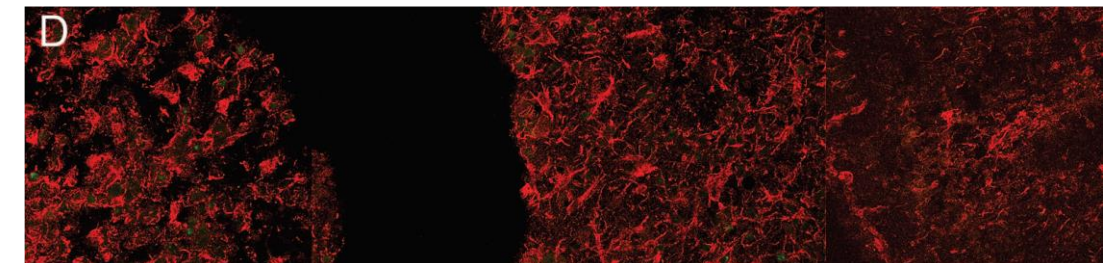
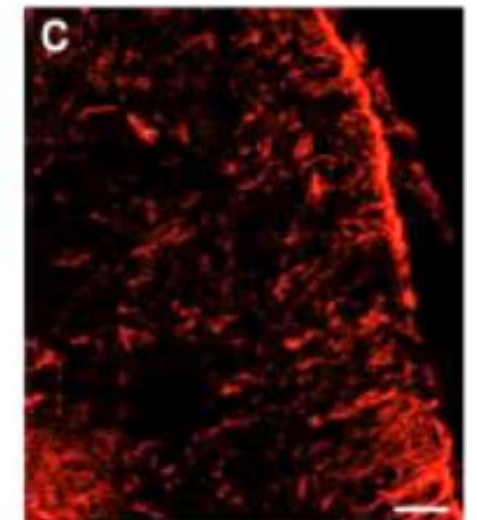
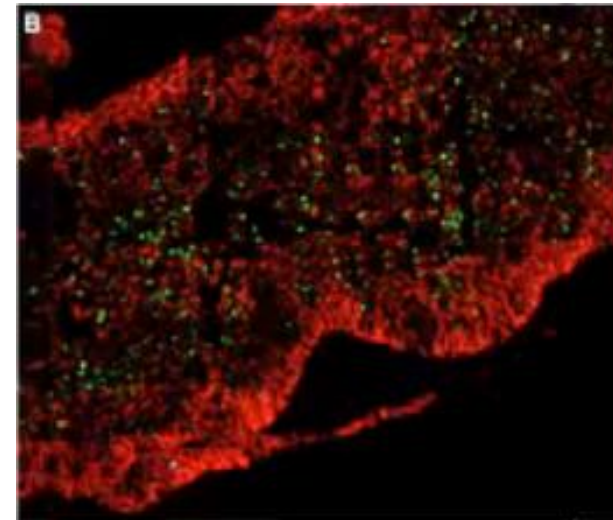
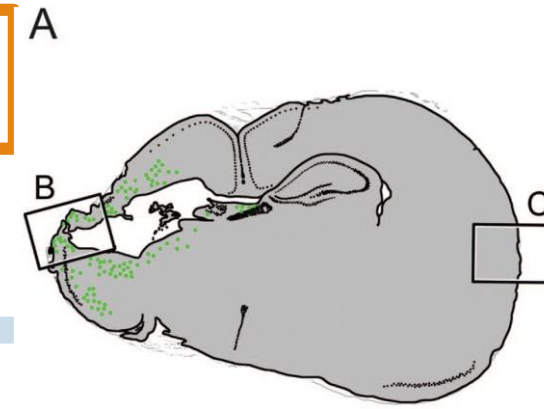
- *Carola Meier et al, Pediatr Res 59: 244–249, 2006*

Figures in Carola Meier et al

- A. Schematic representation of the distribution of human cells in the lesioned hemisphere.** HLA-DR-positive cells (green) are detected in the area of hypoxic-ischemic lesion. Contralateral hemisphere devoid of human cells.
- B. HLA-DR-positive mononuclear cells (green)** are located within a scaffold of GFAP-(+) astrocytes (red) in the area of the hypoxic-ischemic lesion.
- C. Non-lesioned parts of the brain,** with absence of inflammatory and apoptotic events are devoid of human cells (absence of green fluorescence).
- D. Lesioned areas of the brain** of animals that did not receive transplantation of human cells are devoid of HLA-immunostaining. Note absence of green (HLA-staining) within the red scaffold of GFAP-positive rat astrocytes.

1. Carola Meier et al, *Pediatr Res* 59: 244–249, 2006

Intraperitoneal transplantation of mononuclear hUCB-derived cells resulted in a specific homing of these cells into CNS and incorporation around the lesioned area



Donor & Host Stem Cell Communication

Donor Stem Cells seek out Host Stem Cells (Chemotaxis). They adhere, not fuse, to each other

Donor cell shares corrective genetic codes to damaged host stem cells while Host stem cell shares information of host cell antigens

Both now normal part of same body

Khan showed it in AMI cases

- *Oxygen and oxygenation in stem-cell therapy for myocardial infarction; Mahmood Khan et al; Life Sciences, 2010: 87(9-10)(L 269-274*



EXCHANGING GIFTS

Synergism of HBOT + Stem Cell Therapy

Proliferation of neural stem cells correlates with Wnt-3 protein in hypoxic-ischemic neonate rats after hyperbaric oxygen therapy

Wang, Xiao-Li; Yang, Yu-Jia; Xie, Min; Yu, Xiao-He; Liu, Chen-Tao; Wang, Xia

NeuroReport: October 29th, 2007 - Volume 18 - Issue 16 - p 1753-1756

Hyperbaric oxygen therapy promoted brain cell proliferation. Wnt-3 is closely associated with the proliferation of neural stem cells. We examined whether hyperbaric oxygen promoted neural stem cells to proliferate and its correlation with Wnt-3 protein in hypoxic-ischemic neonate rats. Hyperbaric oxygen therapy was administered 3 h after hypoxia ischemia daily for 7 days. The proliferating stem cells and Wnt-3 protein were examined dynamically in the subventricular zone. Results showed that stem cells proliferated and peaked 7 days after hyperbaric oxygen therapy. Wnt-3 protein increased to the higher levels 3 days after therapy. Linear regression analysis showed that nestin protein correlated with Wnt-3 protein. We propose that hyperbaric oxygen treatment promote stem cells to proliferate, which is correlated with Wnt-3 protein.

HBOT pre-treated Stem Cell therapy + HBOT Neuro. cases do better than similar groups given no pre-treatment HBOT

34 cases: M=13, F=21; Dx: HIE (n=29), Cranioccephalic trauma (n=2), Motor vehicle trauma (n=1), Motor vehicle Pedestrian trauma (n=2) TBI

Group 1: n=18; pre-treated with 10 HBOTs → Stem Cells → 48 more HBOT sessions

Group 2: n=16; Stem Cells → 48 HBOTs

Results:

- Group 1 had faster & better response with the 1st signs of improvement coming after mean 2.5 weeks in GMFM, Speech, Attention, Function
- Group 2 had similar response but after mean 4.3 weeks

“The results may well be due to the synergistic effects of HBO followed by stem cell therapy. It is believed that stem cells are attracted to neural sites with neural sites with increases vascularity and nutrient rich environment which compensates or aids in repair of neurological damage

- *Stem cell therapy and HBO for brain-injured children and cerebral palsy; J L Diaz-Barbosa & F J Morales; Proc. 3rd Int. Symp. For CP an the Brain-Injured Child, July 2003; Fort Lauderdale, FL, USA*

Higher tissue O₂ & Stem Cells in AMI

AMI ← Deprivation of O₂ & nutrients to heart due to blocked coronaries

O₂ required for constant energy demands of contracting heart, role in the regulation of heart function. However, sudden reperfusion oxygenation of the ischemic myocardium can be injurious.

Results from these studies demonstrate the importance of tissue oxygen in the application of stem-cell therapy to treat CAD.

The transfer of gene products from injured cells may explain stem cell functional & phenotypic changes without the need of trans-differentiation into tissue cells.

Transfer of gene products from stem cells may reprogram injured cells to site of injury

- *J. Int. Soc. Nephro, tissues; 2010; 78(9): 838-848*

Requirements of transplanted stem cells



**Land unprepared.
Cant expect a good crop**

Land prepared. Expect a better crop



PATIENT NAME _____	BIRTHDATE _____	PATIENT # _____		
AGE: _____	HISTORY FORM OF: _____	REVIEWED TODAY'S DATE: _____		
Changes in RCG and/or PSH are as follows: _____ No Change				
Constitutional (3 of 7)				
Height _____	Weight _____	Temp _____ Pulse _____ Resp _____ BP _____		
General Appearance _____				
Chief Complaint: _____				
History of Present Illness: note location, qualities, severity, duration, timing, context, modifying factors, associated signs & symptoms				
Problem 1:	New	Established	Stable	Evolving
Problem 2:	New	Established	Stable	Evolving
Problem 3:	New	Established	Stable	Evolving



What is Diabetes?

Diabetes is not a simplified high blood sugar disease. It is a dysfunctional disease of protein, fat and carbohydrate metabolism, endothelial inflammation and other complications in the CVS, CNS, Kidneys, NASH, Foot, DPN, etc.

- Bl. Sugar is just an economic test and we interpolate its results for a guess at what is happening in the WHOLE body.

T1DM: Direct insulin deficiency relative to body needs

T2DM: relative deficiency of insulin activity to metabolise oxydisable foods to release their energy for cell and body needs.

Hyperactivity of antagonistic / counteracting hormones like excessive thyroxine, steroids, somatostatin, catecholamine, etc.

Correct treatment mandates a correct etio-pathological diagnosis and a broad spectrum approach as per need.

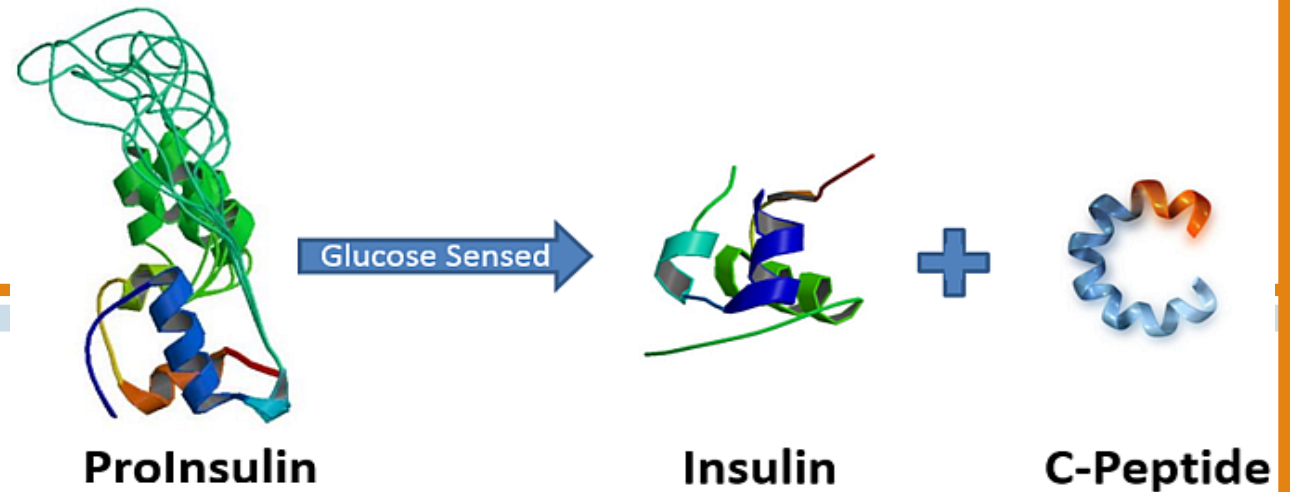
C-Peptide and Insulin

T1DM due to premature burn out of pancreatic β -Cells

Cause: Auto-immunity, viral infection, genetic susceptibility, toxins

Selection: If patients have Fasting C-Peptide < 0.5 , FPG > 200 mg/dl, PP > 300 mg/dl, often in spite of optimum available therapies

- One OHA that helps to an extent is metformin. It reduces Insulin dosage.
- Controlled need-based diet and lifestyle.
- HBOT starts becoming helpful.
- Life long blood sugar monitoring at frequent intervals and Insulin injections are PAINFUL as only a T1DM patients knows, that, too, a child.



Improving C-Peptide in T1DM

When Insulin requirements > 0.5 IU/Kg body weight along with other findings as mentioned above, consider SCT under Helsinki protocol, if the parents demand, as off-label experimental therapy.

Options

- Diet, life style, controlled exercise, metformin, coenzyme Q-10, L-Carnitine
- HBOT + SCT
- Adjuvant drugs to enhance cellular regeneration & energy output

With Regenerative Therapies, C-Peptide may creep towards or even reach normal, by 1 year in quite a few.

Typical T1DM Long Term Follow-up

*Hasan, 20 yrs

August 2015

- HBA1c = 8.2%
- C-Peptide = 0.5 (N = 0.5 to 3.5)
- Anti-Diabetics used Insulin 41 U,

Mid 2017

- Initiated oral Anti-Diabetics
- HBA1c on 10/6/17 = 5.6%
- C-peptide on 10/8/17 = 1.5
- Insulin 32 U

T2DM: relatively easy to treat with OHAs

Metformin; Broad spectrum insulin resistance reducing agent, esp. at liver and adipose tissue.

- Cardio-protective. Some role in NASH.
- All patients need it if tolerated

Pioglitazone: Similar to metformin as an insulin resistance reducer hypoglycemic agent.

- May be tried with full precaution, in patients without pre-existent heart disease,

Sulfonylureas: Used for increasing insulin RELEASE, NOT production, if B-cells (C-Peptide) ok

Repaglinide: Release insulin by a different pathway, used in patients with irregular meal times

Alfa glucosidase inhibitors: Reduce glucose absorption from intestines, in cases who will not stop overeating

Gliptines: Pancreatic protector; reduce apoptosis in pancreas, for all types of T2DM

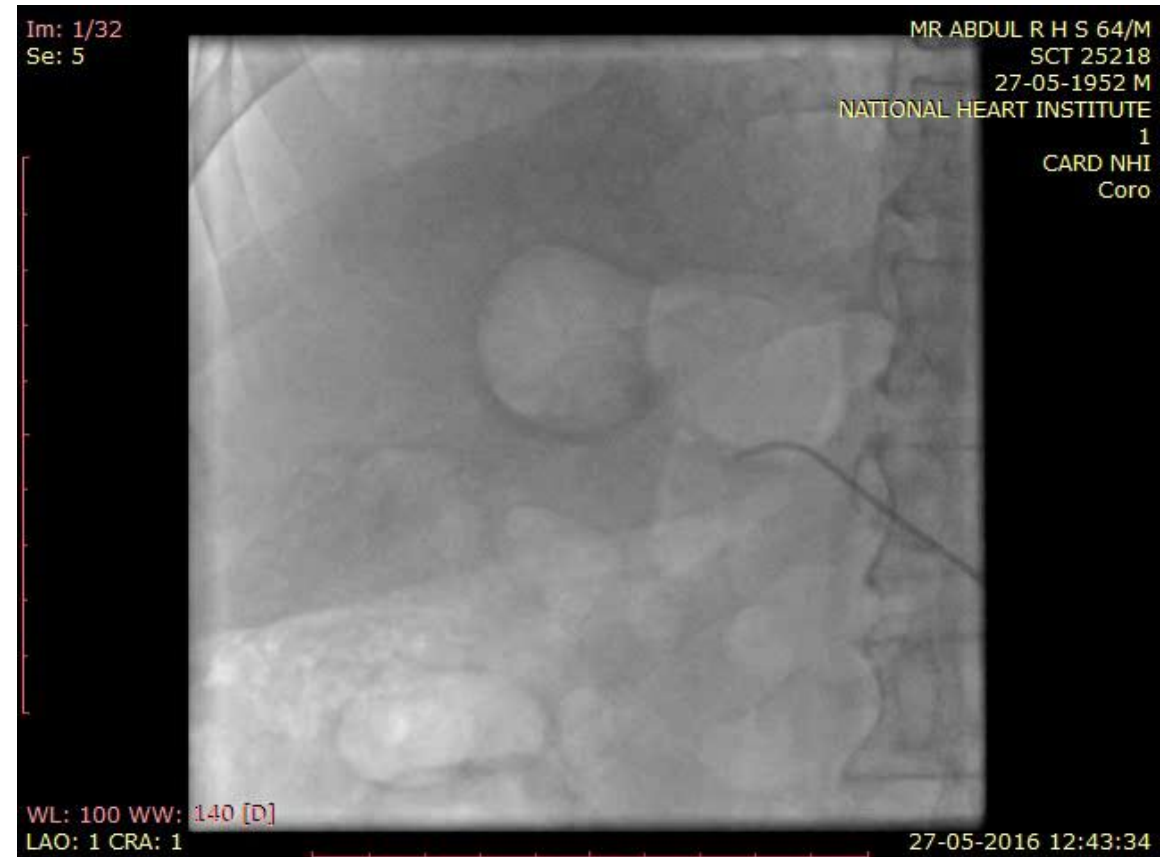
Glifozines: Enhance glucose excretion by kidneys, in cases who will not stop overeating

SCT Therapy in DM 1 & 2 from our series. Procedure common, but when, what, dosage, route: THAT is the key

SCT TO PANCREAS



SCT TO LIVER



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Our Ultimate aim: Help as a Physician should

Dream it

- Do it
- Demonstrate how



Thank
you