OZONE THERAPY IN PATIENTS WITH VIRAL HEPATITIS "C" A CLINICAL STUDY

BY

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Aim of the Study

- To evaluate the effectiveness of ozone therapy in hepatitis C genotype 4 infection.
- To evaluate a proposed ozone therapy protocol in HCV genotype 4 treatment
- This is meant to be a provisional study to be followed by another study

Why HCV?

- Worldwide medical problem (estimated that more than 300 millions suffering from HCV)
- Major medical problem in EGYPT (postulated that more than 15% i.e. more than 10 millions of the population are suffering from HCV)
- Slowly progressing, detected mainly accidentally, devitalizing and difficult to treat

Why HCV? (cont.)

- In most cases it leads to complications e.g. liver cirrhosis, ascitis, liver carcinoma and ultimately liver cell failure
- Not only a medical problem, but also an economic problem (less work, less production and very high costs of usual treatment)

Hepatitis "C"

- There is a worldwide prevalence of genotypes 1,2 & 3.
- In Africa genotype 4 and 5 are more dominant.
- In Asia genotype 6 is more dominant

Genotype differences have shown varying susceptibility to antiviral therapy.

Hepatitis "C" (cont.)

- Liver Cirrhosis is estimated to develop in 20 -25 % of patients with HCV within 20 years.
- Hepato-cellular carcinoma (5% of patients)
- Hepatic decompensation and liver cell failure with ascites

Hepatitis "C" " (cont.)

- The main line of treatment nowadays for hepatitis C include interferon and ribavirin.
- *Ribavirin and interferon have significant medical and psychiatric side effects.*

Why Ozone?

- Known Anti-viral action
- Safe if used by experts
- Less costs
- Classic method of treatment very expensive with major side effects and minimal cure results

Mode of Action of Ozone

Mode of Action of Ozone

- Inactivation of bacteria, viruses, fungi and protozoa:
  - Disrupts the integrity of bacterial cell envelope through oxidation of the phospholipids and lipoproteins.
  - Damages viral capsid and upsets reproductive cycle by disrupting virus-to-cell contact with peroxidation.
  - Inhibits cell growth at certain stages in fungi

(V. Bocci & R. Viebahn)

Mode of Action of Ozone (cont.)

- Enhancement of Circulation:
  - Reduce clumping of red cells and restore its flexibility and oxygen carrying ability.
  - Arterial oxygen partial pressure increases and viscosity decreases leading to better tissue oxygenation
  - Oxidizes plaque in arteries allowing removal of the breakdown products.

(V. Bocci & R. Viebahn)

Mode of Action of Ozone (cont.)

- Stimulation of oxygen metabolism:
  - Increases red blood cell glycolysis rate → stimulation of 2,3-diphosphoglycerate (2,3-DPG) → increase of oxygen released to tissues.
  - Activates Krebs cycle by enhancing oxidative carboxylation of pyruvate → stimulating production of ATP.

  (V. Bocci & R. Viebahn)

Mode of Action of Ozone (cont.)

- Stimulation of the production of the enzymes which act as a free radical scavengers and cell wall protectors:Glutathione Peroxidase, Catalase, and Superoxide Dismutase

- Dissolution of malignant tumors: Ozone inhibits tumor metabolism, oxidizes the outer lipid layer of malignant cells and destroys them through cell lysis

Mode of Action of Ozone (cont.)

- An inducer of cytokines (other oxidizing agents in appropriate amounts induce the synthesis of cytokines in monocytes and lymphocytes)
- H2O2 crosses the cell membrane and activates the cytoplasmic gene-regulatory nuclear factor kappa B, ultimately causing the transcription of mRNAs of several cytokines, namely interleukin (IL-1,2,4,6,8,10), tumor necrosis factor (TNF-$\propto$) and interferon (IFN β and γ) (V. Bocci 2002)

Ozone and HCV

- Lipid and protein peroxides, produced in small amounts by ozonation, have demonstrable antiviral properties.
- Ozone tends to stimulate leucocyte function and cytokine production.
- Ozone increases the oxygen saturation (P02) in erythrocytes and enhances their pliability so that capillary circulation is facilitated (Gérard & Sunnen 2001).

Ozone and HCV (cont.)

- In HCV, viral load appears to be a major factor in the invasiveness and virulence of the disease process.
- Preliminary research has shown that reduction of viral load in Hepatitis C by means of ozone therapy can significantly normalize hepatic enzymes and improve measures of global patient health (Gérard & Sunnen 2001).

Antiviral effect of Ozone

- Denaturation of virions through direct contact with ozone. Ozone disrupts viral envelope proteins, lipoproteins, lipids, and glycoproteins.
- The presence of numerous double bonds in these unsaturated molecules makes them vulnerable to the oxidizing effects of ozone.

Antiviral effect of Ozone (cont.)

- Molecular architecture is disrupted and widespread breakage of the envelope ensues.
- Deprived of an envelope, virions cannot sustain nor replicate themselves.

Antiviral effect of Ozone (cont.)

- Ozone proper, and the peroxide compounds it creates, may directly alter structures on the viral envelope which are necessary for attachment to host cells. Peplomers, the viral glycoproteins protuberances which connect to host cell receptors are likely sites of ozone action.

Antiviral effect of Ozone (cont.)

- Alteration in peplomer integrity impairs attachment to host cellular membranes foiling viral attachment and penetration.
- Ozone induces the release of cytokines by leucocytes .
- Stimulation of the immune mechanisms will lead to significant reduction of circulating virions (Gérard & Sunnen 2001).

PATIENTS and METHODS

Patients and Methods

- Sixty patients type 4 HCV
  - 45 males
  - 15 females
- Age range 34 to 65 years
- Randomly selected

Patients and Methods (cont.)

- Investigations
  - C.B.C, Liver function tests, AFP
  - Prothrombin time and concentration
  - Antibodies for Bilharziasis
  - PCR quantitative for HCV
  - Abdominal ultrasonography

Patients and Methods (cont.)

- Ozone Treatment Protocol:
  - MAH + RI three times per week for eight weeks followed by two times per week for sixteen weeks
  - The rationale of start low and go slow was respected

Patients and Methods (cont.)

- MAH 25µ/ml ozone ↳ 25 ↳ 30 ↳30 ↳35 ↳ 35 ↳ 40 ↳ 40 ↳ 45 ↳ 45 ↳ 50 ↳ 50 ↳55 ↳ 55 ↳ 60 …….. The volume was constant 150 ml
- Rectal Insufflations 20µ/ml ozone x 300 ml ↳ 20µ/ml x 300 ml ↳ 25µ/ml x 300 ml ↳ 25µ/ml x 300 ml ↳30µ/ml x 300 ml ↳ 30µ/ml x 300 ml ↳ 35µ/ml x 300 ml ↳ 35µ/ml x 300 ml ↳ 35µ/ml x 350 ml ↳ 35µ/ml x 350 ml ↳ 40µ/ml x 350 ml ……..

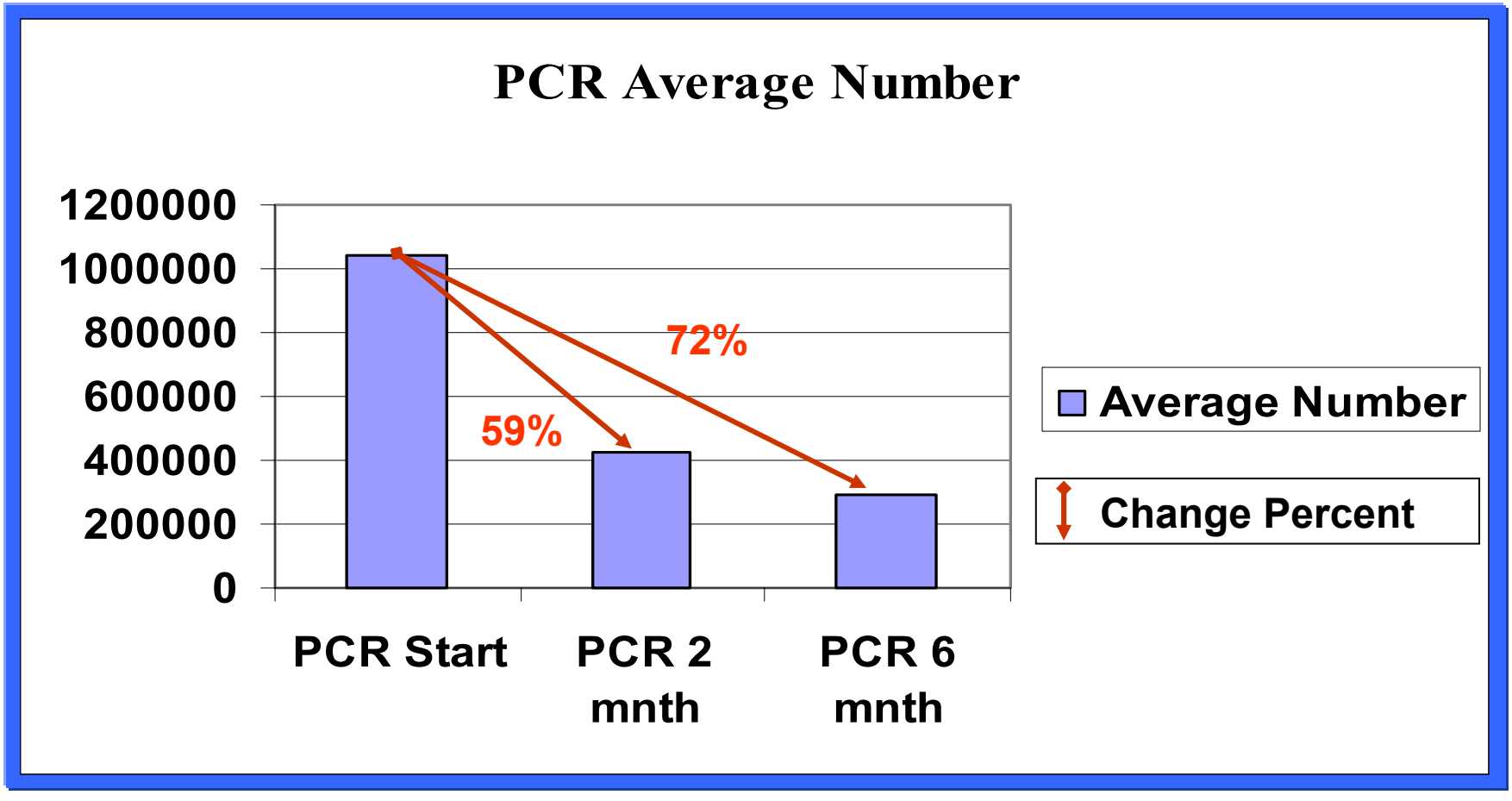
Patients and Methods (cont.)

- Investigations were repeated after 8 and 24 weeks of treatment (but in this study we are focusing on PCR quantitative and Liver enzymes)
- General health and daily activity were observed

RESULTS

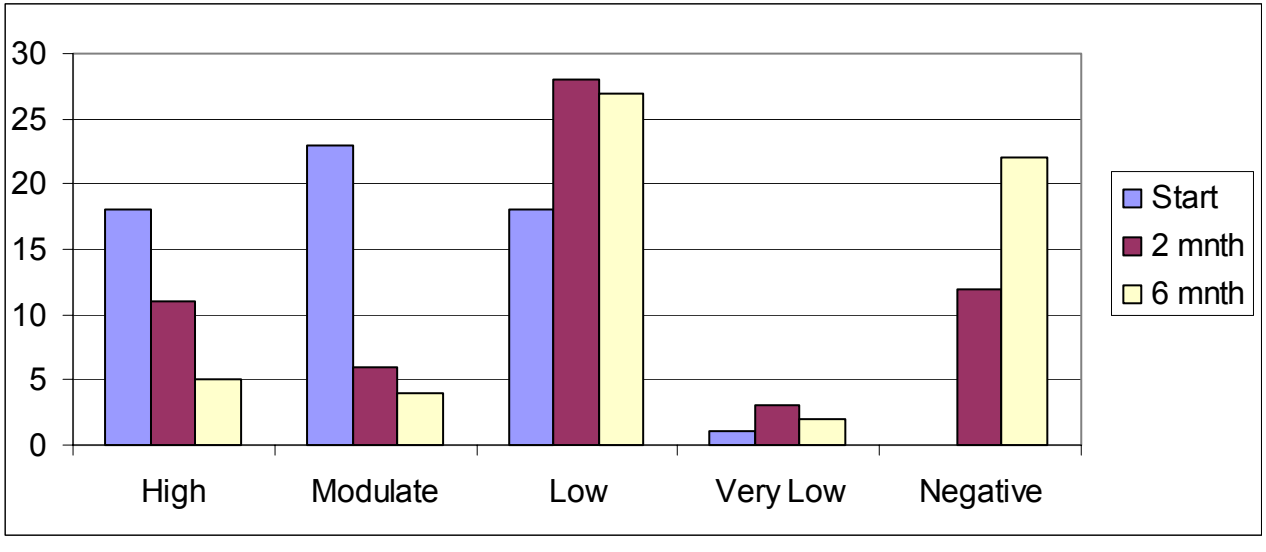
PCR Average Number

Start	1041354
After 2 Months	423215
After 6 Months	293150



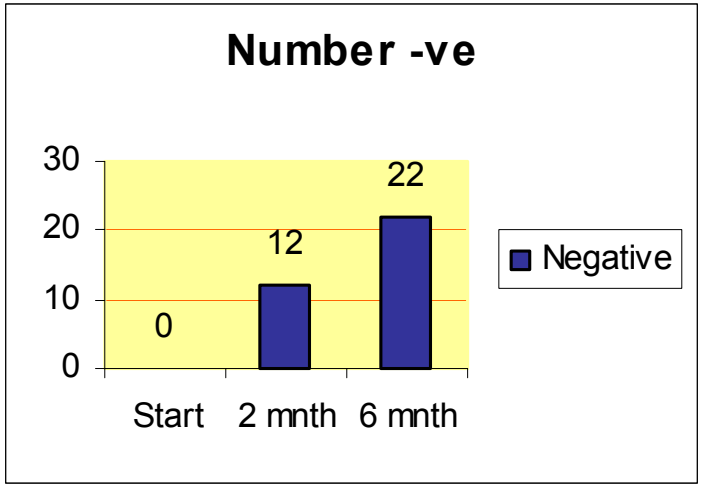
Viral Load Category Change

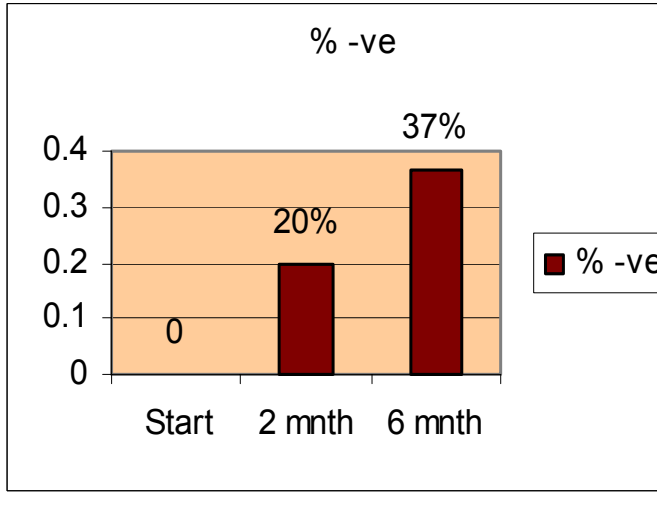
	High > 200,000	Moderate 50,000 : 200,000	Low 2000 : 50,000	V. Low 0 : 2000	Negativ 0
Start	18	23	28	1	0
After 2 Months	11	6	28	3	12
After 6 Months	5	4	27	2	22



PCR % -ve

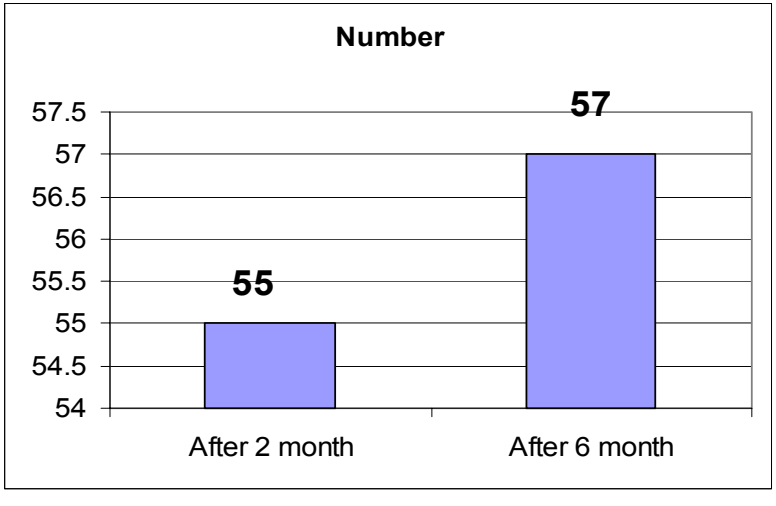
	Number -ve	% -ve
After 2 Months	12	%20.00
After 6 Months	22	%37.67

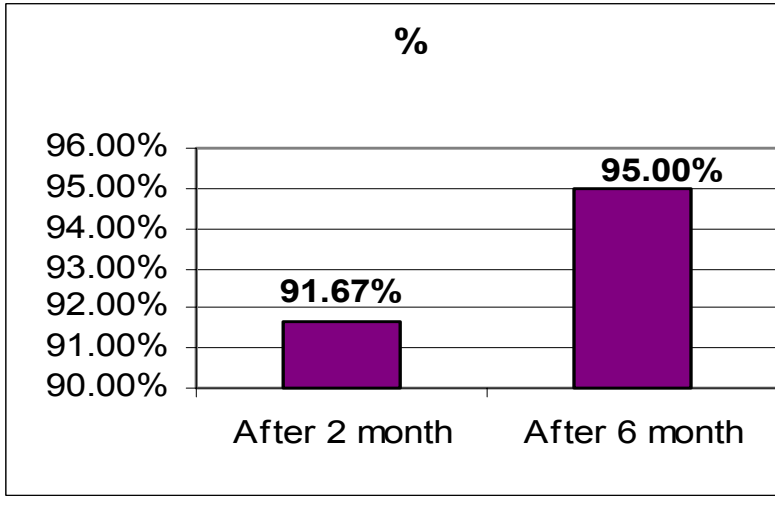




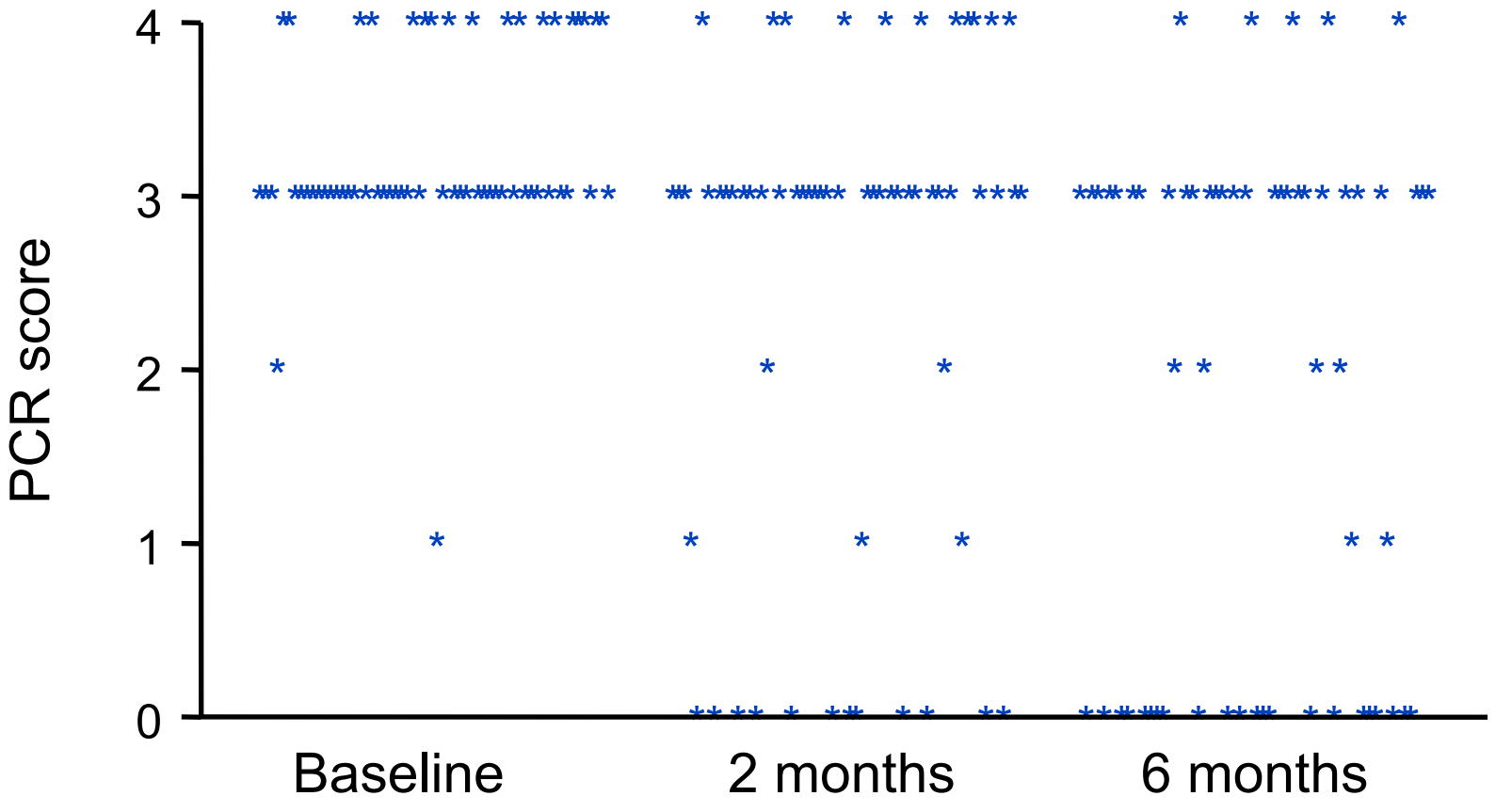
PCR % decline

	Number	%
After 2 Months	55	%91.67
After 6 Months	57	%95.00



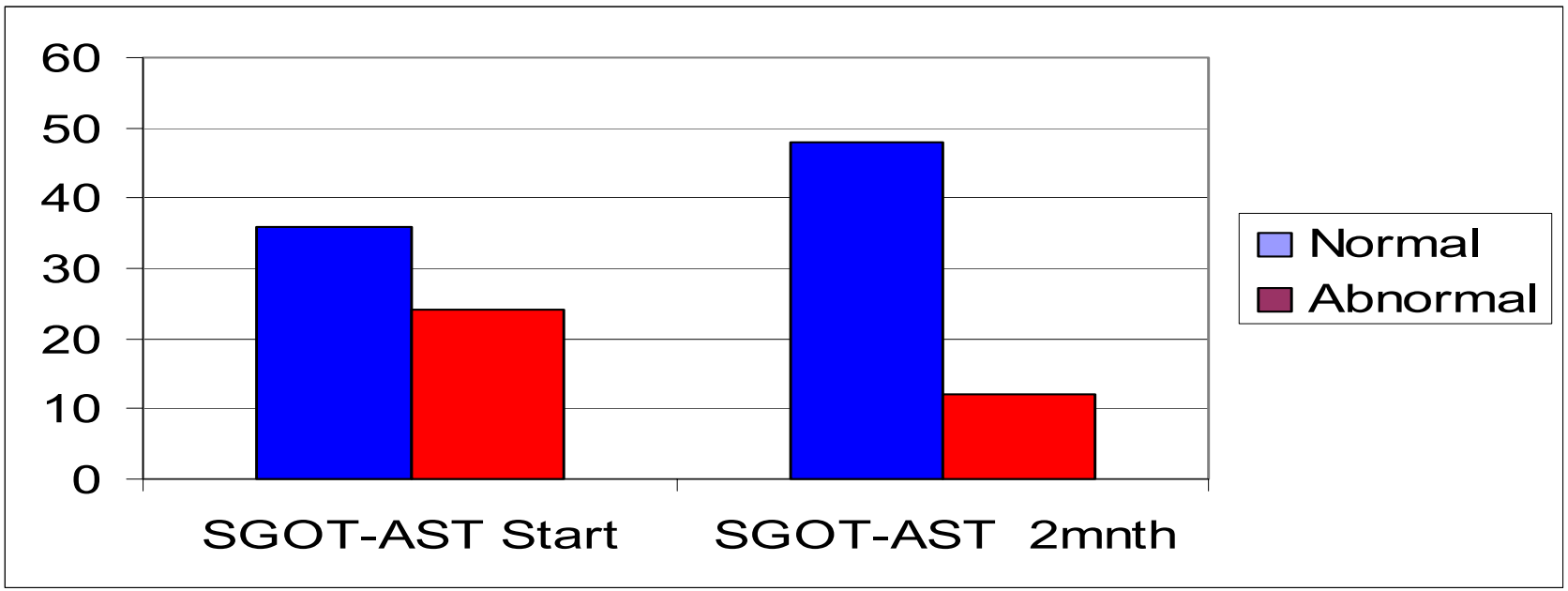


Viral Load Category Change

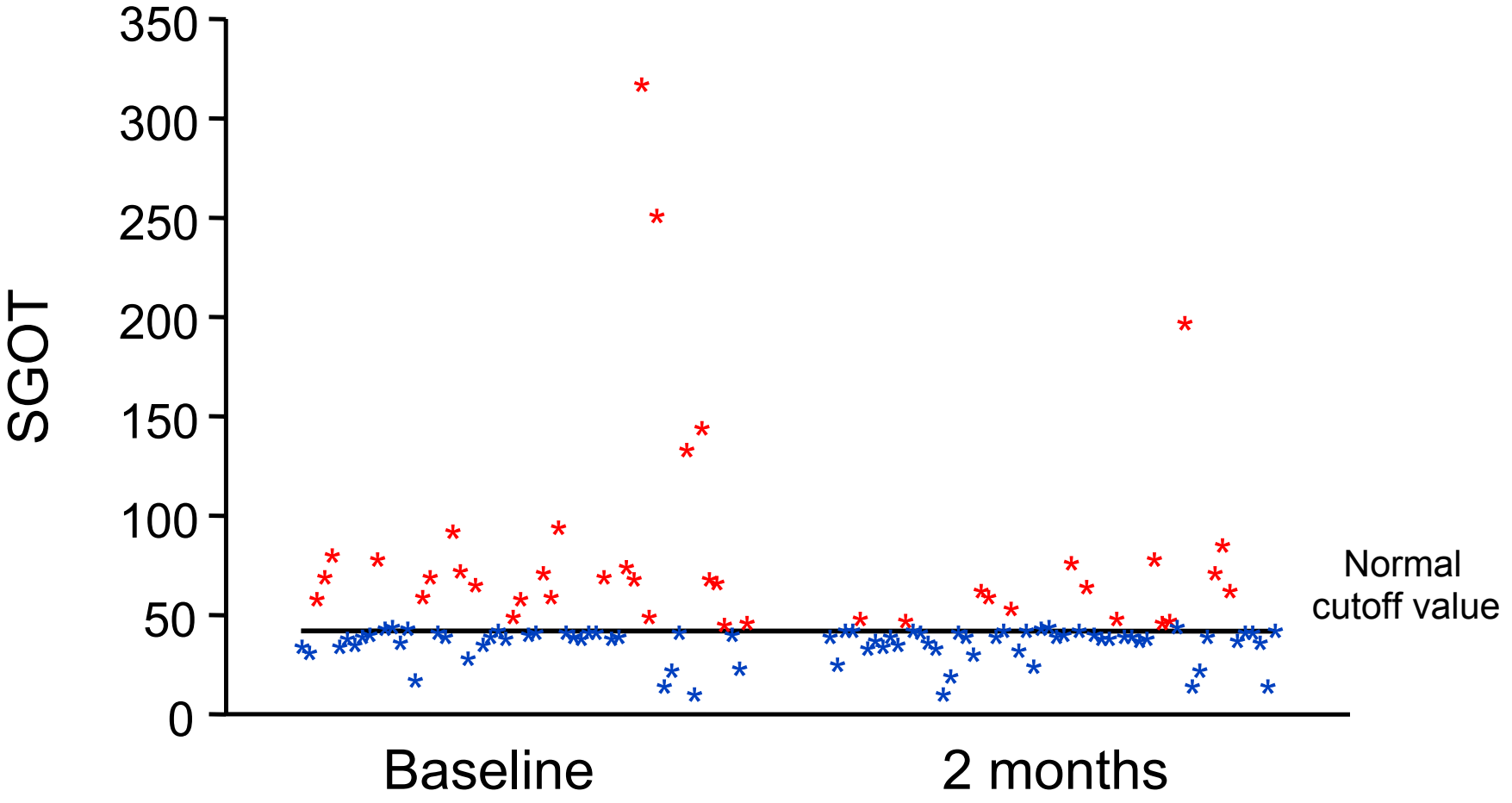


SGOT - AST

Number	Start	2 Month
Normal	36	48
Abnormal	24	12

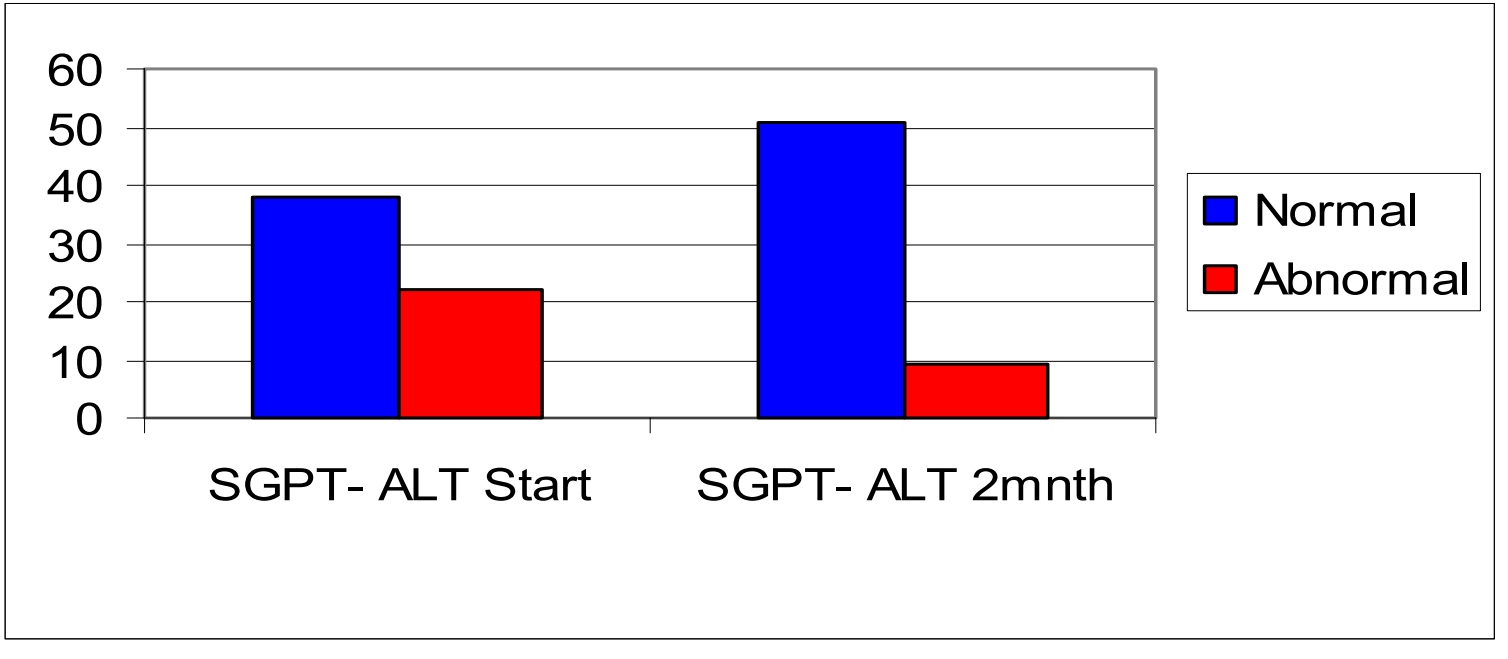


SGOT - AST

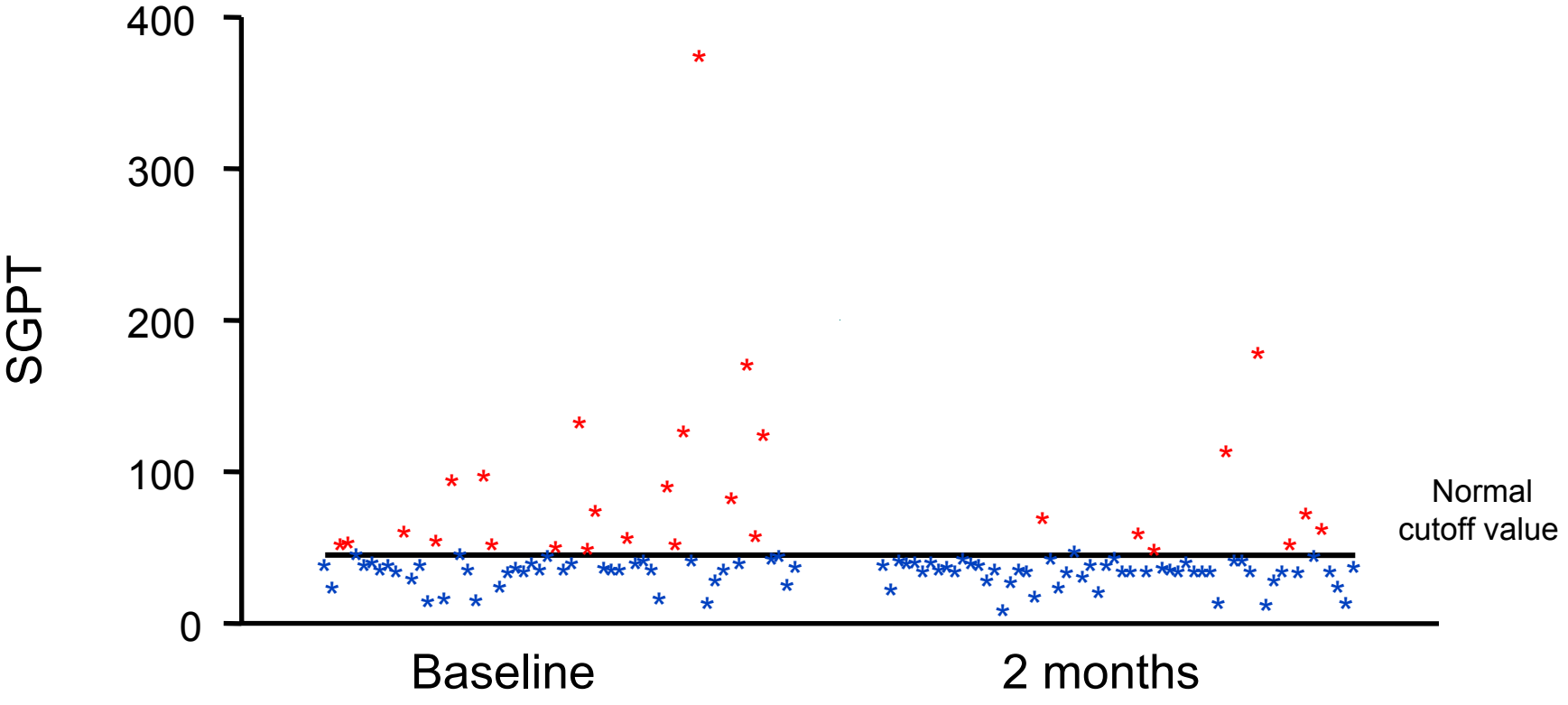

SGOT
350
300
250
200
150
100
50
0
Baseline
2 months
Normal
cutoff value

SGPT - ALT

Number	Start	2 Month
Normal	38	51
Abnormal	22	9



SGPT - ALT



DISCUSSION and CONCLUSION

As a preliminary study Ozone therapy was found to be an effective, safe and less expensive method in Hepatitis "C" patients. But further studies are important

Pilot Studies

- MAH twice/week for 2 months
- MAH three times/week for 2 months
- RI twice/week for 2 months
- RI three times /week for 2 months

Following rationale of start low and go slow

Good results but not as good as the mentioned protocol

Pilot Studies (cont.)

- Combined MAH & RI once /week following 2 months of mentioned protocol was not satisfactory from the general condition point of view
- but if we shift to twice / week the general condition is better.

Comments

- Why Combined MAH & RI ?

  * pilot studies

  * hyper-oxygenation of portal circulation (Knoch et al 1987)

- Why the enzymes are normal in some patients before ozone therapy ?

  * Medications affecting the enzymes level

Important Postulated Reasons for Less Effectiveness

- Diet
- Exertion
- Hepatotoxic Drugs

Recommendation for Further Study

- Double Blind Randomized Placebo Controlled Study
- Selection of Patients
  - No complications (Cirrhosis, Ascitis, Liver cell failure, etc..)
  - No associated chronic disease (Diabetes, Bilharziasis, etc..)

Recommendation for Further Study (cont.)

- Long term study for one year
- Follow-up by observation and investigations for another year
- Evaluation should be based on many parameters
  - General condition
  - Liver Function tests (synthesis,excretions,integrity)

Recommendation for Further Study (cont.)

- Quantitative PCR as one only parameter for evaluation can be considered as a guide for evaluation but is not conclusive
  - sharp fluctuations of viral load
  - so far there is no precise and accurate method for quantitative estimation of viral load
  - different methods, different units and wide variation from one laboratory to another must be put in consideration

Thank You