## S-P-5

### Nitrite/Nitrate Levels in Patients after Stem Cell Transplant

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**Background:** Nitric oxide (NO) is a bio-molecule involved in many diseases such as rheumatoid arthritis, atherosclerosis, ischaemic heart disease and carcinogenesis. NO is synthesized from the oxidation of L-arginine by three NO synthase (NOS) isoforms: constitutive endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS). Nitric oxide production has been shown to be one of the important mediators in immunological response to stimuli. At high levels, it may contribute to host immune defense, but at the same time may also be responsible for tissue damage at the target organs.

**Materials and Method:** Thirty-nine randomly selected patients (20 males and 19 females: aged 17-67 years, mean age 37.9 years) undergoing stem cell transplant (SCT) at Queen Mary Hospital were included in this study from June 1999 to April 2000. Plasma nitrite/nitrate ($NO_2^-/NO_3^-$) levels were measured by a chemiluminescence analyzer (Sievers 280 NO analyzer ) after de-proteinization.

**Results:** In this study, we have shown that the plasma nitrite and nitrate ($NO_2^-/NO_3^-$), the stable end-products of nitric oxide (NO), were significantly increased in human acute graft-versus-host disease (GVHD) following allogeneic sibling (SIB) or matched unrelated donor (MUD) stem cell transplantation (SCT). Patients with acute had significantly ($p<0.001$) higher maximum levels of plasma $NO_2^-/NO_3^-$ (159.9± 29.1microM) when compared with the baseline levels (19.5± 1.4microM), as well as the maximum levels of patients developed no acute GVHD (49.8± 3.8microM). Moreover, there was a good correlation between plasma $NO_2^-/NO_3^-$ levels and the clinical severity of acute GVHD. There was a good time correlation between the rise of the plasma $NO_2^-/NO_3^-$ and the clinical onset of acute GVHD.

**Conclusion:** These findings support that nitrite and nitrate may be one of the important mediators in the pathogenesis of acute GVHD following human stem cell transplantation. It may therefore be useful as a clinical marker for diagnosis.

## S-MM-1

### Quantitative PCR for Diagnosis of alpha Thalassemia

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**Background:** alpha thalassemia (thal) is the commonest genetic disease in SE Asia, due to deletion of alpha globin genes on Chromosome 16. Couples with the loss of 2 alpha globin genes in cis on the same chromosome (alpha° thal carrier) have a 25% chance of a homozygous child (loss of 4 alpha globin genes) with each pregnancy. This fetus (Hb Barts hydrops fetalis) is incompatible with life and mothers have severe complications in late gestation. Prenatal testing for homozygous alpha° thal fetuses relies on the detection of alpha genes in fetal DNA. Whilst PCR amplification of alpha gene fragment has been advocated as a highly sensitive and rapid procedure, its specificity is comprised by the possibility of contamination with maternal or other DNA template and PCR failure due to allelic dropout, thus the more labor-intensive Southern blotting technique has remained the method of choice in many service laboratories.

**Patients and Method:** We devised a quantitative PCR (Q-PCR) method based on the TaqMan technology. Primers and TaqMan probe were designed with the help of the Primer Express Software (PE Biosystems Inc.) to specifically amplify an alpha° thal chromosome fragment or a normal chromosome fragment respectively. Variations in input target DNA in individual sample wells were normalized by the simultaneous amplification of the beta-actin gene fragment under limiting primers condition.

**Results** : The ratio of alpha° thal: bate-actin was significantly different for homozygous alpha° thal (Hb Barts hydrops), heterozygote alpha° thal and normal subjects, with mean ± SD ratios of 1.483± 0.343, 0.377± 0.126 and 0.000± 0.0 respectively in each group. Similarly, mean ratio of normal chromosome fragment:beta-actin was different in the three groups with no overlap in individual data. Study of 30 at risk pregnancies using this Q-PCR technique compared favourably with the result of conventional Southern hybridization. The effect of DNA contamination was also tested and up to 10% of maternal DNA contamination did not affect the specificity of the result.

**Conclusion:** Q-PCR is a reliable and rapid method for α thal prenatal diagnosis.