**Statistical analysis plan for the Hyperbaric Oxygen in Lower Limb Trauma (HOLLT) randomised controlled trial**

Principal Investigator: Dr Ian Millar

Project Coordinator: Dr Rosemary McGinnes

Supervising Statistician: Professor Rory Wolfe

ACTRN12607000559415 (<http://www.anzctr.org.au/trial_view.aspx?ID=81796>)

NCT00264511 (<http://clinicaltrials.gov/show/NCT00264511>)

NHMRC Project Grant 490966

Drafted 9 Sept 2015: Rory Wolfe,

Last updated: 30 September, 2015

Final Edit: Ian Millar

**Hypothesis**

A course of hyperbaric oxygen (HBO) will reduce the rate of short term and long term complications of lower limb injury involving tibial fracture with severe soft tissue injury and that this reduction in complications will improve functional and quality of life outcomes for patients.

**Data acquisition**

Study data collectors at each site review the patient, take digital photographs of the injured leg where possible, access pathology test results, examine the clinical record and access radiological images to gather a data set that is forwarded, with digital copies of wound photography and radiology images, to the Coordinating Centre.

**Primary outcome**

The primary outcome measure is the incidence of a wound complication after injury. This is a composite measure defined as the occurrence within 2 weeks of injury of: (i) significant soft tissue necrosis developing after the initial surgery, or (ii) significant wound infection. Wounds were assessed at approximately day 14, or when the patient was discharged from hospital if discharge occurred earlier.

▸ Significant soft tissue necrosis is determined by the amount of tissue surgically debrided after the initial surgery. Minimal trimming of skin edges removing no more than a few millimetres of tissue to clean the surface of a wound which was not obviously necrotic will be considered normal surgical practice and will not be scored as indicating ‘significant necrosis’.

▸ Wound infection occurring during the acute phase is defined according to USA Centre for Disease Control guidelines for assessing surgical wound infections.

Clinicians not involved in the patient’s care and blinded to the identity and location of the patient, and the allocation to HBO or no-HBO group, will adjudicate if necrosis or infection is present and classify type of infection. In cases of disagreement a consensus decision is reached as to whether significant wound infection is present.

A second adjudication process for the primary outcome will use guidelines aimed at generating a “clinically relevant” definition of endpoint. This was determined to be necessary in retrospect as clinical practice across sites varied with respect to treatment of necrosis and infection more than was originally thought. (See Appendix 2 for guidelines for adjudicating clinicians)

**Secondary outcomes**

*Day 14 outcomes:*

Amputation, need for fasciotomy, time until bone coverage and until skin closure, breakdown of closed wounds, time until definitive orthopaedic fixation, number of operative procedures, length of stay in ICU, length of stay in the acute hospital.

*Radiological and clinical outcomes at 3, 6, 9, 12, 18 and 24 months:*

Radiological union is determined by blinded review of X-rays taken at approximately 3, 6, 9, and 12 months, and at 18 months and 2 years in cases not united at 12 months. Review and scoring of radiographs is performed by assessors who are blinded to the trial group allocation and the time at which the radiographs were taken post-injury. Clinical adjudications are performed by two independent orthopaedic surgeons.

Outcomes are the occurrence of: delayed or non-union (a bone graft having been undertaken, or use of non-standard adjuncts such as electrical stimulation), soft tissue infection (prolonged courses of antibiotics and/or debridement surgery), osteomyelitis, any other wound complications, whether the patient is able to bear weight, and the number and nature of any other surgical procedures that have been required.

*Functional and quality-of-life outcomes at 12 months and 2 years:*

Return-to-work (component of the Sickness Impact Profile), disability (global disability scale), pain (visual analogue scale), SF36 physical and mental quality of life (from a language-appropriate version of the questionnaire), lower limb injury score (lower limb injury component of the Short Musculoskeletal Functional Assessment).

**Statistical Analysis**

Primary analyses will be by intention to treat, i.e. according to randomised treatment allocation, and will take the form of unadjusted comparisons of each outcome between the two treatment groups. A two-sided test p-value less than 0.05 will be considered statistically significant with no adjustment made to p-values for the assessment of the multiple secondary outcomes since they are pre-specified.

The primary outcome will be summarised as a proportion of patients who experience the endpoint and these proportions will be compared between groups using a Pearson’s chi-square test. A secondary analysis of the primary outcome will use mixed-effects logistic regression to adjust for: (i) Gustilo grade 1/2/3a/3b/3c as a fixed effect with contiguous categories being combined if any of the four grade categories has fewer than 10 patients or fewer than 5 events, and (ii) recruiting centre as a random effect with centres that recruited fewer than 10 patients being combined as a single “other” centre (to avoid instability in the model estimation procedure). Adjustment will subsequently also be made for any major baseline imbalance (amounting to the equivalent of a 0.25 standard deviation difference in means of a baseline continuous variable or an odds ratio of 1.5 for a baseline binary variable with prevalence at least 5%).

A secondary analysis of the primary outcome will use the second “clinically relevant” definition of endpoint adjudication. The methods for this analysis will be identical to those for the original definition of the primary outcome.

Secondary outcomes that are binary, e.g. amputation, fasciotomy, will be analysed using the same methods as for the primary outcome. Questionnaire outcomes will be compared between groups using a t-test for primary analysis and using linear regression for secondary analyses following the same structure of unadjusted and adjusted analyses used for the primary outcome. The distribution of questionnaire outcomes across the measurement scale will be assessed and, if appropriate, the outcome will be treated as an ordered categorical variable and analysed using ordered logistic regression with testing of the proportional odds assumption. Number of procedures will be analysed using Poisson regression with over-dispersion tested for by comparing the model’s fit with a negative binomial model. Time to event outcomes, including length of stay (which can be conceptualised as time to discharge), will be analysed using Cox proportional hazards regression with testing of the proportional hazards assumption.

Analysis will be undertaken to explore the relationship between acute complications and long term functional and QoL outcomes.

Subgroup analyses will be undertaken by Gustilo grade, specifically high-grade fractures (Gustilo grade 3b and 3c) versus lower grades (Gustilo grade 1, 2 and 3a) and by recruiting centre. For Gustilo grade these subgroup analyses will use the relevant regression models and include an interaction between randomised treatment group and fracture grade 3b/3c versus 1/2/3a. For recruiting centre the subgroup analysis will involve inclusion of an interaction as a random effect between randomised treatment group and centre with small centres combined as before.

In addition to Gustilo grading, the data set may enable injury grading according to other commonly reported severity grading systems such as MESS score, AO grade of fracture, host score for predisposition to infection. If such alternative injury severity scoring proves feasible, then further secondary analyses will adjust for these gradings in place of Gustilo.

Per-protocol analysis of the primary outcome and selected secondary outcomes will be conducted according to treatment received, with a minimum of six completed HBOT sessions to be considered as a therapeutic course of HBO therapy.

Missing data is only a minor concern for primary outcome or Day 14 outcomes since missing outcomes are anticipated for at most approximately half a dozen participants, and hence complete case analyses will be applied for these outcomes. The same argument applies, and the complete case analysis approach will be taken, for late complications analysed at 12 months. For quality of life and functional outcomes at the later time points the analysis strategy will assume that missing data are missing at random and an assessment of the sensitivity to this assumption will be undertaken.

**Proposed structure for a main paper to summarise the HOLLT trial findings**

Figure 1: CONSORT diagram of patient recruitment and retention

Table 1: Description of baseline characteristics of the two randomised groups

Table 2: For patients receiving HBO therapy, details of HBO sessions provided including any side effects or complications. For patients on usual case, description of any HBO intervention received.

Table 3: Primary and selected secondary outcomes results of primary analyses. Results of secondary analyses adjusted for Gustilo grade. If relevant, results of secondary analyses adjusted for Gustilo grade and factors displaying imbalance at baseline.

Table 4: Sub-group analysis by Gustilo grade.

**Appendix 1**

Extract from HOLLT Protocol, version 8.1 2011.

11.3 Basis of Sample Size and Power

Revision of the sample size to 120 subjects followed a review of the viability of the HOLLT Study as a result of slow recruitment. An assessment of the blinded outcome data available for the first 40 patients showed an event rate of 51.2% which is higher than expected and a doubling of the event rate on which the study was powered.

The original sample size of 250 subjects was selected to provide 80% power to detect a reduction in the incidence of a defined set of acute complications from 30% to 15% at p=0.05. Selection of 30% as the estimated control complication rate was based upon our pilot data and is mid-range with respect to published complication rates for the injuries targeted. Whilst a 10% or even lesser absolute reduction in acute complications would be sufficient to justify system wide adoption of a simple medical or surgical intervention, we believe that a 15% absolute reduction in complications is a more appropriate evidence target given the overall aim of determining whether it is appropriate to build new trauma centre hyperbaric facilities, given the logistical complexity and cost involved.

The original protocol noted that Bouachour’s randomised and blinded study reported an even more powerful benefit than our original target assumed – Bouachour found a reduction in the requirement for further surgery from 33% to 6%, albeit with small sample size and therefore significant uncertainty. We would therefore hope that a statistically significant improvement in clinical outcomes would be demonstrable with lesser numbers.

The timing and nature of interim analyses will be determined by the DSMC taking into account any advice provided by the PMG. The study viability review of unblinded data from 40 subjects found a substantially higher number of identified complications, suggesting that either the patients enrolled actually do suffer more complications that assumed, or more likely, that our measures are more sensitive or have a lower threshold for defining complications. Regardless of which factor is responsible, the DSMC and Project Management Group assessed this as giving the study greater power to identify a significant difference with a reduced overall enrolment target.

Given that 30% is also representative of the rates of poor late outcomes such as failure to return to work, requirement for late surgery for osteomyelitis or non-union and presence of significantly reduced quality of life, our original target sample size of 250 subjects was assessed as sufficient to allow detection of late outcome improvements if a significant effect exists. With the reduction in the planned sample size to 120 subjects, more substantial differences in outcomes would need to occur and be measurable for the study to demonstrate late outcomes benefit, if these do in fact exist, or else our measures will need to be more sensitive. With a broad range of late outcome measures, of both the categorical and continuous variable type, it is hoped the latter may be the case.

**Appendix 2**

**Guidelines for Clinician Adjudicators regarding Secondary Analysis of HOLLT necrosis and infection outcomes**

The following guidelines are applicable to clinicians blinded to intervention group who are undertaking the secondary analysis of the HOLLT Primary Outcome. The Secondary analysis is aimed to identify all cases where there is at least a reasonable probability of some necrosis and/or infection having occurred, with these stratified to those of little clinical consequence, versus those that are “clinically significant”. This secondary analysis is intended to provide a more inclusive identification of the possible occurrence of infection or necrosis than the more objective but more limited “a-priori” definitions in the HOLLT Protocol, but to then refine these so as to identify those that are would be judged to be of significant clinical consequence by a practising orthopaedic trauma surgeon.

***Adjudication Guidelines:***

***Infection:***

**Probable but minor infection**

Any evidence of infection, including observer opinion of “probable” infection, or opinion of “possible” infection with supporting evidence of minor infection such as treatment with a short course of oral antibiotics or appearances of cellulitis, pin site infection, pus etc. but not meeting criteria for Clinically Significant Infection

**Clinically significant infection**

Infection leading to necrosis of soft tissue, requiring debridement or washout, removal of fixation, re-opening of wound, a change of surgical plan or associated with a prolonged inpatient stay for management of infection or its complications. Any case where intravenous antibiotics prescribed, or where oral antibiotics continued for a longer period than required for incidental and minor soft tissue infection (suggested in excess of 10 - 14 days). Any case where possible or probable infection of any severity is identified in the first 14 days which is then followed by deep infection at follow up as evidenced by debridement or removal of prosthetic material or finding of infection at subsequent surgery and where it seems likely that the late infection was a sequel of early infection.

***Necrosis:***

**Minor necrosis**

Any evidence of soft tissue necrosis occurring within the first 14 days, including when recorded as a data point, when recorded in notes or evidenced by photographic appearance consistent with necrosis, BUT – not reaching criteria for Clinically Significant Necrosis

**Clinically significant necrosis**

Any necrosis scored as “moderate” or “severe” according to previously used a-priori, Primary Outcome assessment guidelines (i.e. based upon extent of surgical debridement), OR – any case where necrosis is judged to have been the indication for a prolonged hospital stay for conservative wound management and/or where debridement of necrosis has been undertaken on the ward or in clinic, or any case where there is evidence of necrosis that is complicated by or caused by infection.