BMJ Open

Preoperative single-dose methylprednisolone versus placebo after major liver resection in adults: protocol for a randomised controlled trial

Alexsander K Bressan,[1] Derek J Roberts,[1,2] Sana U Bhatti,[1] Elijah Dixon,[1] Francis R Sutherland,[1] Oliver F Bathe,[1] Chad G Ball[1]

To cite: Bressan AK, Roberts DJ, Bhatti SU, *et al*. Preoperative single-dose methylprednisolone versus placebo after major liver resection in adults: protocol for a randomised controlled trial. *BMJ Open* 2015;**5**: e008948. doi:10.1136/bmjopen-2015-008948

► Prepublication history for this paper is available online. To view these files please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2015-008948).

Received 1 June 2015
Revised 3 August 2015
Accepted 21 August 2015

[1]Department of Surgery, University of Calgary and the Foothills Medical Centre, Calgary, Alberta, Canada
[2]Division of Epidemiology, Department of Community Health Sciences, University of Calgary, Calgary, Alberta, Canada

Correspondence to
Dr Chad G Ball;
Ball.Chad@gmail.com

ABSTRACT

Introduction: Although randomised controlled trials have demonstrated that preoperative glucocorticoids may improve postoperative surrogate outcomes among patients undergoing major liver resection, evidence supporting improved patient-important outcomes is lacking. This superiority trial aims to evaluate the effect of administration of a bolus of the glucocorticoid methylprednisolone versus placebo during induction of anaesthesia on postoperative morbidity among adults undergoing elective major liver resection.

Methods and analysis: This will be a randomised, dual-arm, parallel-group, superiority trial. All consecutive adults presenting to a large Canadian tertiary care hospital who consent to undergo major liver resection will be included. Patients aged <18 years and those currently receiving systemic corticosteroid therapy will be excluded. We will randomly allocate participants to a preoperative 500 mg intravenous bolus of methylprednisolone versus placebo. Surgical team members and outcome assessors will be blinded to treatment allocation status. The primary outcome measure will be postoperative complications. Secondary outcome measures will include mortality, the incidence of several specific postoperative complications, and blood levels of select proinflammatory cytokines, acute-phase proteins, and laboratory liver enzymes or function tests on postoperative days 0, 1, 2 and 5. The incidence of postoperative complications and mortality will be compared using Fisher's exact test, while the above laboratory measures will be compared using mixed-effects models with a subject-specific random intercept.

Ethics and dissemination: This trial will evaluate the protective effect of a single preoperative dose of methylprednisolone on the hazard of postoperative complications. A report releasing study results will be submitted for publication in an appropriate journal, approximately 3 months after finishing the data collection.

Trial registration number: NCT01997658; Pre-results.

Strengths and limitations of this study

- This randomised controlled trial will evaluate the effect of a single preoperative dose of methylprednisolone on the incidence of postoperative complications and other patient-important outcomes instead of surrogate outcome measures.
- Surgical team members, participants, and outcome assessors will be blinded to study allocation status.
- This is a single-centre study.

INTRODUCTION

The acute-phase response, first described in 1930,[1] constitutes the pathophysiological reaction to tissue injury. This response begins locally through upregulation of a complex network of cytokines, and then generalises to the systemic level, likely through the combined actions of interleukin-1 (IL-1), tumour necrosis factor-α (TNF-α) and IL-6.[2–5] Of these three cytokines, IL-6 has consistently been reported to correlate with the magnitude of tissue injury and expected systemic inflammatory response. This cytokine stimulates expression of hepatic acute phase proteins and release of anti-inflammatory mediators, including IL-10,[6] and adrenal cortex steroids. After surgery, IL-6 represents a reliable marker of the extent of surgical trauma[7–10] and an independent predictor of postoperative morbidity.[11 12]

The magnitude of the acute phase response to elective liver resection is not particularly greater than that after other complex abdominal surgeries.[13] However, greater morbidity seems to result from the systemic inflammatory response associated with postoperative liver dysfunction.[14 15] For instance, prolonged

use of vascular clamping techniques during liver transection is associated with heightened systemic inflammation,[16] ischaemic liver injury and liver dysfunction.[17] The resultant insufficient hepatic synthesis of acute phase proteins and coagulation factors may result in impaired immune defence against bacterial infections[18] and an increased risk for postoperative bleeding.

Glucocorticoids are potent anti-inflammatory drugs that modulate expression of both inflammatory and anti-inflammatory genes.[19] Over the last decade, randomised controlled trials (RCTs) testing preoperative administration of glucocorticoids in patients undergoing liver resection have demonstrated favourable postoperative changes in laboratory markers of systemic inflammation, including IL-6, IL-10, TNF-α, C reactive protein (CRP), and liver enzymes and function tests,[20–23] such as aspartate amine transferase (AST), alanine amine transferase (ALT), prothrombin time (PT) and total bilirubin. To date, no increased risk for infection has been associated with preoperative use of glucocorticoids in this population.[23] Direct negative effects of glucocorticoids on liver regeneration were also not demonstrated after liver resection in a study with rats.[24]

Although the above studies have demonstrated that preoperative glucocorticoids may improve surrogate outcomes among patients undergoing major liver resection, evidence supporting improved postoperative patient-important outcomes is lacking. A recent systematic review of randomised clinical trials, which included five studies that enrolled a total of 379 patients, was unable to demonstrate a significant difference in hospital length of stay or postoperative morbidity between glucocorticoid and control groups.[25] However, this study was limited by the low number of RCTs, small sample sizes and considerable clinical heterogeneity between the included studies.

Objective

The objective of this RCT is to determine the efficacy of a single preoperative dose of methylprednisolone for reducing the incidence of postoperative complications in adult patients undergoing major liver resection.

METHODS AND ANALYSIS

Overview

This will be a single-centre, parallel-group RCT. We will randomly allocate adult patients to receive either an intravenous single dose of methylprednisolone (500 mg) or placebo (saline) during induction of anaesthesia for major elective liver resection. We hypothesise superiority of the intervention for reducing the incidence of postoperative complications.

Study setting

This trial will be set at the Foothills Medical Centre (FMC) in Calgary, Alberta, Canada. The FMC is a University of Calgary-affiliated tertiary care centre, which performs approximately 200 liver resections in-house per year. This hospital offers a fellowship training programme in hepatobiliary surgery recognised by the Americas Hepato-Pancreato-Biliary Association (AHPBA), and is staffed by four fellowship-trained HPB surgeons, all of whom will participate in the study.

Patient population and eligibility criteria

The population will consist of all consecutive adult patients at the FMC who consent to undergo elective major liver resection, defined as planned resection of three or more liver segments. Indications for surgery will include both primary and secondary hepatic malignancies and benign liver pathologies. Patients aged <18 years and currently receiving systemic therapy with glucocorticoids or unable to provide informed consent will be excluded from the study. Combined biliary reconstruction, vascular reconstruction and other simultaneous extrahepatic procedures do not constitute exclusion criteria.

Recruitment

All adult patients scheduled to undergo elective liver resection at the FMC will be assessed for enrolment at least 2 weeks before surgery. Eligible patients will be contacted by telephone and those who are interested in participating will be sent a copy of the consent form by mail. A trained research assistant will inform patients about the voluntary nature of participation in the trial, and the involved risks and benefits. Signed consent forms will be returned by mail or in person on the day of surgery.

Randomisation, allocation concealment and blinding

Once consent is provided, participants will be assigned by the research assistant to either the experimental or control group according to a random allocation sequence. An online random list generator will be utilised to perform blocked randomisation in a 1:1 ratio. Block size will not be disclosed to preserve allocation concealment.

The anaesthetist will receive an opaque sealed envelope containing the patient allocation status and assigned medication immediately before induction of anaesthesia. The medication container, total volume to be infused and technique of administration will be the same for active and control treatments.

Participants, outcome assessors, surgical team members, care providers (except the anaesthesiologist) and data analyst will be blinded to study allocation status.

Intervention

During induction of anaesthesia, the intervention will be administered as a 500 mg single dose of methylprednisolone (Solu-Medrol, Methylprednisolone Sodium Succinate for Injection USP, Pfizer) over 15–20 min.

Surgical conduct during and after liver resection

Perioperative care, including use of epidural analgesia (during and after surgery), antibiotic prophylaxis, type of incision, use of hepatic pedicle clamping (technique and duration), transfusion of blood components, use of drains and prophylaxis for deep venous thrombosis will be provided to both intervention groups at the discretion of the attending surgeon.

Data collection

A research assistant will collect data elements from patient medical records, including:

Demographic data:

- Date of birth;
- Gender;
- Preoperative diagnosis;
- Previous treatments;
- Comorbid conditions;
- Baseline laboratory tests: AST, ALT, PT, CRP, albumin, total bilirubin and fractions;
- The American Society of Anesthesiologists' (ASA) classification for preoperative health assessment of surgical patients.[26]

Operative information:

- Use and scheme of perioperative antibiotic prophylaxis;
- Use of epidural anaesthesia;
- Type of incision;
- Participation of fellow, resident or medical student/clinical clerk in the surgical team;
- Extent of liver resection;
- Types of concomitant extrahepatic surgery;
- Use of hepatic pedicle clamping (technique and duration);
- Use of surgical drains;
- Estimated blood loss;
- Transfusion of blood products (packed red blood cells, platelets, fresh frozen plasma, albumin) and number of units;
- Volume of intravenous crystalloid infusion;
- Duration of surgery (defined as the number of hours between skin incision and closure of skin).

Postoperative complications

An independent board-certified general surgeon will be the outcome assessor. He will be instructed about diagnostic criteria for specific postoperative complications in this trial. In conjunction with the surgical team, the outcome assessor will evaluate all participants on a daily basis during hospitalisation. On hospital discharge, all patients will receive orientation about signs and symptoms of complications, and will be encouraged to contact their surgeon's office in case of any concerns related to postoperative recovery.

After hospital discharge, participants will be assessed for postoperative complications in the outpatient clinic approximately 30 and 90 days after surgery. Outcome assessor and the surgical team will be blinded to the study allocation status of participants.

Diagnostic criteria for specific postoperative complications:

- Liver failure: defined according to the International Study Group of Liver Surgery criteria.[27]
- Ascites: confirmed by imaging, and if symptomatic or requiring paracentesis.
- Intra-abdominal fluid collection: confirmed by imaging, and if symptomatic or requiring percutaneous or surgical drainage.
- Infected intra-abdominal fluid collection: further defined by turbid output through surgical or percutaneous drain and need for antibiotic therapy.
- Bile leak: any evidence of bile output through the surgical drain or following postoperative percutaneous drainage.
- Haemorrhage: if requiring reoperation, radiological or endoscopic intervention; information on postoperative transfusion of blood products (type and number of units) will also be collected.
- Pleural effusion: if symptomatic or requiring thoracocentesis or drainage.
- Pulmonary embolism: confirmed by V/Q scan or CT.
- Deep venous thrombosis: confirmed by Doppler-ultrasonography or cross-sectional imaging.
- Renal failure: if requiring dialysis.
- Arrhythmias: any change in cardiac sinus rhythm prompting specific medical intervention or patient transfer to a monitored bed.
- All cases of acute myocardial infarction, cerebrovascular accident or transient ischaemic attack, as diagnosed by appropriate medical specialist.
- Other infectious complications (pneumonia, urinary tract infection (UTI), bloodstream infection and surgical site infection): defined by the Centers for Disease Control and Prevention diagnostic criteria.[28–31]
- Other: any unlisted postoperative complications requiring specific medical treatment, radiological intervention or reoperation.

Laboratory analyses

During induction of anaesthesia, 12 mL of blood will be drawn via venipuncture into a BD Vacutainer Blood Collection Tube (Bencton, Dickinson and Company) with clot activator gel. Blood samples will be spun within 15 min at 700×g at room temperature. Plasma will be aliquoted from each sample and stored at −80°C until analysis. Stability for up to 2 years is demonstrated for cytokines stored under this temperature.[32] Additional blood samples will be collected on postoperative days 1, 2 and 5 following the same procedure, for serial postoperative laboratorial evaluation.

Cytokine concentration will be determined in all plasma samples using Bio-Plex Pro^{T} Human Cytokine 8-Plex Assay (Bio-Rad Laboratories) and Luminex MAGPIX technology. Luminex offers multiplexed cytokine measurements based on colour-coded magnetic

microspheres with comparable sensitivity to traditional enzyme-linked immunoassay-based systems. MAGPIX platform combines utilisation of light-emitting diodes to illuminate all beads at once, and a digital camera to capture several images for analysis. Higher throughput, a smaller amount of fluid requirement and reduced costs have been reported.[33]

Preoperative values of laboratory liver tests (AST, ALT, PT/international normalised ratio (INR) and total bilirubin) and CRP performed within the last month before surgery will be used as baseline values (postoperative day 0). Additional blood samples will be collected on postoperative days 1, 2 and 5 for serial laboratory evaluation.

Study outcomes

Primary outcome measure:

- Incidence of postoperative complications within the 90-day period after liver resection.

Secondary outcomes:

- Hospital length of stay after elective liver resection.
- Ninety-day postoperative mortality; percentage of deaths among all participants within the 90-day period after liver resection.
- Incidence of specific postoperative complications within the 90-day period after liver resection.
- Blood level of select inflammation-associated cytokines (IL-6, TNF-α and IL-10) during induction anaesthesia, and on postoperative days 1, 2 and 5.
- Blood level of C reactive protein, liver enzymes and function tests (AST, ALT, PT/INR and total bilirubin) at baseline (preoperative evaluation), and on postoperative days 1, 2 and 5.

Statistical methods

Sample size calculation

Considering the incidence of postoperative morbidity of 37.8% in the control group and 13.9% in the steroid group,[23] and an estimated dropout rate of approximately 20%, 75 participants will be required in each study arm to achieve statistical power (superiority trial) of 80% with an α level of 5%. Considering the current volume of major liver resections at FMC, data collection is expected to be completed in a 24-month period.

Participants with an intraoperative finding of additional hepatic or extrahepatic disease precluding liver resection will be excluded from outcome analysis. Data from all other randomised participants will undergo an intention-to-treat analysis.

Descriptive statistics

Continuous variables will be summarised using means and SDs (normally distributed data), or medians and ranges (non-normally distributed data). We will summarise categorical data using counts, proportions and risk ratios with 95% CIs.

Planned outcome analysis

Primary outcome:

- The incidence of overall postoperative complications will be compared using Fisher's exact test.

Secondary outcomes:

- Hospital length of stay: expected positively skewed frequency distribution of data will be evaluated in histograms, and summary measures of median and IQR will be reported for each study group. Hypothesised reduction in hospital length of stay in favour of the steroid group will be evaluated using Mann-Whitney U test.
- Comparison of 90-day postoperative mortality between study groups will be performed using Fisher's exact test.
- Incidence of specific complications will be reported as counts and percentage of 90-day overall morbidity for each study group. Fisher's exact test will be utilised to compare percentage of specific complications between study groups. Considering the expected low number of events for each specific complication, comparison of all infectious complications (surgical site infection, infected intra-abdominal fluid collection, pneumonia, UTI and blood stream infection) between study groups will also be performed.
- We will compare the results of laboratory tests (IL-6, IL-10, TNF-α, AST, ALT, PT and total bilirubin) over time using mixed-effects models with a subject-specific random intercept.[34 35] Model covariates will include the intervention group, time postrandomisation, and an interaction term between intervention group and time postrandomisation. These models are a validated method for analysing clustered data over time, and can better handle missing data than the alternate methods (such as generalised estimating equations).[36 37]

We hypothesise superiority of glucocorticoid compared with standard of care in reducing the incidence of postoperative complications and we will use statistical hypothesis test to investigate the evidence for this. We will only utilise two-tailed tests, and significance level will be set at p value <0.05. All statistical analyses will be conducted using Stata/SE V.12.0 (Stata Corp LP, College Station, Texas, USA) and R V.3.0.1 (available at http://www.r-project.org/).

DISCUSSION

This clinical trial will build on the rationale already established by previous studies, replicating a reportedly safe administration scheme for the intervention and aiming to further evaluate the effect on the incidence of postoperative complications as the main outcome measure.

Defining the expected beneficial effect in this term represents a fundamental step to translate the pathophysiology knowledge accumulated over the last decade into current routine clinical practice.

Ethics and dissemination

Research ethics approval

This study has received approval from the Conjoint Health Research Ethics Board (REB13-0294) at the University of Calgary. The study protocol, informed consent form and other submitted documents were reviewed and approved.

Confidentiality

On recruitment, the research assistant will give a unique scrambled study number to each participant. Only the study number will be used to identify participants. Data collection sheets and any printout of electronic files will be kept in a locked filing cabinet in a secure office with limited access. The master list of participants and informed consent forms will be securely stored separately from de-identified participant records. All digital files will be password protected and stored in a firewall-protected secure environment.

Dissemination policy

Final results will be publicly disseminated regardless of the magnitude or direction of the intervention effect on study outcomes. A report releasing study results will be submitted for publication in an appropriate journal, approximately 3 months after finishing data collection.

Twitter Follow Derek Roberts at @DerekRoberts01

Acknowledgements DJR is supported by an Alberta Innovates—Health Solutions Clinician Fellowship Award, a Knowledge Translation Canada Strategic Training in Health Research Fellowship, and funding from the Canadian Institutes of Health Research and the Clinician Investigator Program at the University of Calgary.

Contributors CGB is the principal investigator and together with ED, FRS and OB has coordinated and actively participated in all the phases of trial design, statistical analysis plan and drafting of the protocol. SUB designed the data collection form. CGB, SUB and AKB submitted the application to the ethics board. AKB wrote the main manuscript. DJR critically revised the design, methodology, statistical analysis plan and the main manuscript. All authors contributed to the writing of the manuscript and agreed with submission of the final version for publication.

Competing interests AKB is the current Hepato-Pancreato-Biliary Fellow of the Department of Surgery at the University of Calgary. DJR is a surgery and Clinician Investigator Program Resident as well as a Doctor of Philosophy (Epidemiology) candidate at the University of Calgary. SUB is a former Clinical Research Fellow of the Department of Surgery at the University of Calgary. CGB, ED, FRS and OB are academic hepatobiliary and pancreatic surgeons at the Foothills Medical Center.

Ethics approval The study protocol for this clinical trial has been approved by the Conjoint Health Research Ethics Board (CHREB) at the University of Calgary.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1. Tillett WS, Francis T Jr. Serological reactions in pneumonia with non-protein somatic fraction of pneumococcus. *J Exp Med* 1930;52:561–71.
2. Baigrie RJ, Lamont PM, Kwiatkowski D, *et al*. Systemic cytokine response after major surgery. *Br J Surg* 1992;79:757–60.
3. Castell JV, Gomez-Lechon MJ, David M, *et al*. Interleukin-6 is the major regulator of acute phase protein synthesis in adult human hepatocytes. *FEBS Lett* 1989;242:237–9.
4. Biffl WL, Moore EE, Moore FA, *et al*. Interleukin-6 in the injured patient. Marker of injury or mediator of inflammation? *Ann Surg* 1996;224:647–64.
5. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* 1999;340:448–54.
6. Steven M, Opal MD, Vera A, *et al*. Anti-inflammatory cytokines. *Chest* 2000;117:1162–72.
7. Shenkin A, Fraser WD, Series J, *et al*. The serum interleukin 6 response to elective surgery. *Lymphokine Res* 1989;8:123–7.
8. Pullicino EA, Carli F, Poole S, *et al*. The relationship between the circulating concentrations of interleukin 6 (IL-6), tumor necrosis factor (TNF) and the acute phase response to elective surgery and accidental injury. *Lymphokine Res* 1990;9:231–8.
9. Cruickshank AM, Fraser WD, Burns HJ, *et al*. Response of serum interleukin-6 in patients undergoing elective surgery of varying severity. *Clin Sci (Lond)* 1990;79:161–5.
10. Ohzato H, Yoshizaki K, Nishimoto N, *et al*. Interleukin-6 as a new indicator of inflammatory status: detection of serum levels of interleukin-6 and C-reactive protein after surgery. *Surgery* 1992;111:201–9.
11. Szczepanik AM, Scislo L, Scully T, *et al*. IL-6 serum levels predict postoperative morbidity in gastric cancer patients. *Gastric Cancer* 2011;14:266–73.
12. Wen XH, Kong HY, Zhu SM, *et al*. Plasma levels of tumor necrotic factor-alpha and interleukin-6, -8 during orthotopic liver transplantation and their relations to postoperative pulmonary complications. *Hepatobiliary Pancreat Dis Int* 2004;3:38–41.
13. Wiezer MJ, Meijer C, Vuylsteke R, *et al*. Is major liver surgery associated with an increased systemic inflammatory response? A prospective comparison of hemihepatectomy and other major abdominal surgery. *Liver* 1999;19:220–7.
14. Balzan S, Belghiti J, Farges O, *et al*. The “50–50 criteria” on postoperative day 5: an accurate predictor of liver failure and death after hepatectomy. *Ann Surg* 2005;242:824–8, discussion 8–9.
15. Mullen JT, Ribero D, Reddy SK, *et al*. Hepatic insufficiency and mortality in 1,059 noncirrhotic patients undergoing major hepatectomy. *J Am Coll Surg* 2007;204:854–62; discussion 62–4.
16. Man K, Fan ST, Ng IO, *et al*. Tolerance of the liver to intermittent pringle maneuver in hepatectomy for liver tumors. *Arch Surg* 1999;134:533–9.
17. Teoh NC. Hepatic ischemia reperfusion injury: contemporary perspectives on pathogenic mechanisms and basis for hepatoprotection-the good, bad and deadly. *J Gastroenterol Hepatol* 2011;26(Suppl 1):180–7.
18. Fang H, Liu A, Dirsch O, *et al*. Serum LBP levels reflect the impaired synthetic capacity of the remnant liver after partial hepatectomy in rats. *J Immunol Methods* 2012;382:68–75.
19. Barnes PJ. Anti-inflammatory actions of glucocorticoids: molecular mechanisms. *Clin Sci (Lond)* 1998;94:557–72.
20. Yamashita Y, Shimada M, Hamatsu T, *et al*. Effects of preoperative steroid administration on surgical stress in hepatic resection: prospective randomized trial. *Arch Surg* 2001;136:328–33.
21. Schmidt SC, Hamann S, Langrehr JM, *et al*. Preoperative high-dose steroid administration attenuates the surgical stress response following liver resection: results of a prospective randomized study. *J Hepatobiliary Pancreat Surg* 2007;14:484–92.
22. Muratore A, Ribero D, Ferrero A, *et al*. Prospective randomized study of steroids in the prevention of ischaemic injury during hepatic resection with pedicle clamping. *Br J Surg* 2003;90:17–22.
23. Aldrighetti L, Pulitano C, Arru M, *et al*. Impact of preoperative steroids administration on ischemia-reperfusion injury and systemic responses in liver surgery: a prospective randomized study. *Liver Transpl* 2006;12:941–9.
24. Shibata T, Mizuguchi T, Nakamura Y, *et al*. Low-dose steroid pretreatment ameliorates the transient impairment of liver regeneration. *World J Gastroenterol* 2012;18:905–14.
25. Richardson AJ, Laurence JM, Lam VW. Use of pre-operative steroids in liver resection: a systematic review and meta-analysis. *HPB (Oxford)* 2014;16:12–19.
26. Dripps RD. New classification of physical status. *Anesthesiology* 1963;24:111.

27. Rahbari NN, Garden OJ, Padbury R, *et al*. Posthepatectomy liver failure: a definition and grading by the International Study Group of Liver Surgery (ISGLS). *Surgery* 2011;159:713–24.
28. Centers for Disease Control and Prevention. Pneumonia (ventilator-associated [VAP] and non-ventilator-associated pneumonia [PNEU] event. January 2015 (modified April 2015). http://www.cdc.gov/nhsn/PDFs/pscManual/6pscVAPcurrent.pdf
29. Centers for Disease Control and Prevention. Urinary tract infection (catheter-associated urinary tract infection [CAUTI] and non-catheter-associated urinary tract infection [UTI]) and other urinary system infection [USI]) events. January 2015 (modified April 2015. http://www.cdc.gov/nhsn/PDFs/pscManual/7pscCAUTIcurrent.pdf
30. Centers for Disease Control and Prevention. Bloodstream infection event (central line-associated bloodstream infection and non-central line-associated bloodstream infection). January 2015 (modified April 2015). http://www.cdc.gov/nhsn/PDFs/pscManual/4PSC_CLABScurrent.pdf
31. Centers for Disease Control and Prevention. CDC/NHSN protocol corrections, clarification, and additions. April 2013. http://www.cdc.gov/nhsn/pdfs/pscmanual/9pscssicurrent.pdf
32. De JW, Bourcier K, Rijkers GT, *et al*. Prerequisites for cytokine measurements in clinical trials with multiplex immunoassays. *BMC Immunol* 2009;12:52..
33. Baker HN, Murphy R, Lopez E, *et al*. Conversion of a capture ELISA to a Luminex xMAP assay using a multiplex antibody screening method. *J Vis Exp* 2012;(65):e4084.
34. Ware JH. Linear models for the analysis of longitudinal studies. *Am Stat* 1985;14:95–101.
35. Laird NM, Donnelly C, Ware JH. Longitudinal studies with continuous responses. *Stat Methods Med Res* 1992;1:225–47.
36. Liu G, Gould AL. Comparison of alternative strategies for analysis of longitudinal trials with dropouts. *J Biopharm Stat* 2002;12:207–26.
37. Mallinckrodt CH, Clark WS, David SR. Accounting for dropout bias using mixed-effects models. *J Biopharm Stat* 2001;11:9–21.