BMJ Open

The AspiriN To Inhibit SEPSIS (ANTISEPSIS) randomised controlled trial protocol.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2016-013636
Article Type:	Protocol
Date Submitted by the Author:	27-Jul-2016
Complete List of Authors:	eisen, damon; James Cook University Division of Tropical Health and Medicine, Moore, Elizabeth; Monash University, Epidemiology and Preventive Medicine leder, karin; Monash University, Epidemiology and Preventive Medicine Lockery, Jessica; Monash University, Epidemiology and Preventive Medicine mcbryde, emma; James Cook University Division of Tropical Health and Medicine, Australian Institute of Tropical Health and Medicine McNeil, John; Monash University, Epidemiology & Preventive Medicine Pilcher, David; Australian and New Zealand Intensive Care Society Centre for Outcomes and Resource Evaluation, Melbourne, VIC, Australia Wolf, Rory; Monash University, Epidemiology and Preventive Medicine woods, robyn; Monash University, Epidemiology and Preventive Medicine
Primary Subject Heading:	Public health
Secondary Subject Heading:	Infectious diseases, Intensive care
Keywords:	Public health < INFECTIOUS DISEASES, Adult intensive & critical care < INTENSIVE & CRITICAL CARE, Microbiology < BASIC SCIENCES

The AspiriN To Inhibit SEPSIS (ANTISEPSIS) randomised controlled trial protocol.

Damon P Eisen 1,2,3#

Elizabeth M Moore 3

Karin Leder 3,4

Jessica Lockery 3

Emma S McBryde 5#

John J McNeil 3

Dave Pilcher 3,6,7

Rory Wolfe 3

Robyn L Woods 3

1. Townsville Hospital and Health Service

   100 Angus Smith Drive, Douglas QLD 4814 AUSTRALIA

2. College of Medicine and Dentistry

Division of Tropical Health and Medicine

James Cook University Townsville QLD 4811 AUSTRALIA

3. Department of Epidemiology and Preventive Medicine

Monash University, Commercial Rd, Prahran, Victoria, 3184 AUSTRALIA

4. Victorian Infectious Diseases Service at the Peter Doherty Institute

Royal Melbourne Hospital, 792 Elizabeth St, Melbourne, Victoria, 3000 AUSTRALIA

5. Australian Institute of Tropical Health and Medicine

Division of Tropical Health and Medicine

James Cook University Townsville QLD 4811 AUSTRALIA

6. Department of Intensive Care, The Alfred Hospital, Commercial Road, Prahran, Victoria 3004 AUSTRALIA

7. The Australian and New Zealand Intensive Care Society (ANZICS) Centre for Outcome and Resource Evaluation (CORE), Ievers Terrace, Carlton, Victoria 3154 AUSTRALIA

Formerly affiliated with Victorian Infectious Diseases Service at the Peter Doherty Institute, Royal Melbourne Hospital

Telephone: +61 7 4433 2459

Fax: +61 7 4433 1271

Email. damon.eisen@health.qld.gov.au

Keywords: Aspirin, primary prevention, elderly, sepsis, death, hospitalisation.

Word Count: Text 3643

ABSTRACT

Introduction. Sepsis is a leading global cause of morbidity and mortality, and is more common at the extremes of age. Moreover, the cost of in-hospital care for elderly patients with sepsis is significant. There are indications from experimental and observational studies that aspirin may reduce inflammation associated with infection. This paper describes the rationale and design of the AspiriN To Inhibit SEPSIS (ANTISEPSIS) trial, a sub-study of ASPirin in Reducing Events in the Elderly (ASPREE). ANTISEPSIS primarily aims to determine whether low-dose aspirin reduces sepsis-related deaths in older people. Additionally it will assess whether low-dose aspirin reduces sepsis-related hospitalisations and sepsis-related ICU admissions.

Methods and analysis: ASPREE is a double-blinded, randomised, placebo-controlled primary prevention trial that will determine whether daily low-dose aspirin extends disability-free longevity in 19,000 healthy older people recruited in Australia and the US. The ANTISEPSIS sub-study involves additional ASPREE trial data collection to assess the impact of daily low-dose aspirin on sepsis-related events in the 16,703 ASPREE participants aged 70 years and over, recruited in Australia. The intervention is a daily 100 mg dose of enteric-coated aspirin versus matching placebo, with 1:1 randomisation. The primary outcome for the ANTISEPSIS sub-study is the incidence of sepsis-related death in eligible patients. The incidence of sepsis-related hospital and ICU admissions are secondary outcomes. ANTISEPSIS is to be conducted between 2012 and 2018.

Discussion: This sub-study will determine whether aspirin, an inexpensive and accessible therapy, safely reduces sepsis-related deaths and hospitalisations in older Australians. If shown to be the case this would have profound effects on the health of older Australians.

Trial Registration: ANTISEPSIS Australian New Zealand Clinical Trials Registry identifier:

ACTRN12613000349741.

Strengths and limitations of this study

This trial uses the unequivocal measure of death due to sepsis as its primary endpoint.

This large scale, primary prevention, trial will be adequately powered to test the study hypothesis.

There is, however, no opportunity to examine the biological basis for any demonstrated effect, as real-time samples are not available at the time of sepsis episodes.

INTRODUCTION

Joseph Lister pioneered a crucial aspect of modern health practice when he first used carbolic acid solution to prevent severe infection in a contaminated wound. Antisepsis is used in all aspects of health care and daily life to protect us from pathogenic microorganisms. With the AspiriN To Inhibit SEPSIS (ANTISEPSIS) trial, we aim to confirm whether a simple and cheap health intervention can protect against manifestations of severe infection by modulating the human host response to infection, regardless of the specific causative microbe.

Sepsis is a lethal condition that kills one person a minute globally. Lower respiratory tract infections alone, caused 5.5% of all deaths in 2012, making them the third most common cause of death.[1] More specifically, in relation to our study, lower respiratory tract infections are the cause of an extremely high number of sepsis deaths in the elderly in the developed world.[2]

Septic shock has hospital mortality in excess of 40%.[3] Incremental reduction in sepsis mortality has been achieved in high-income countries through early recognition and optimisation of immediate treatment delivered in well-resourced hospitals.[4] However, this reduction mainly parallels general improvements in mortality of patients admitted to ICU.

Sepsis therapy research is bedevilled with failed attempts at proving efficacy of specific treatments.[5] Adjuvant treatments for sepsis that have been developed and trialled at major cost have failed to deliver significant reduction in mortality. There is no effective sepsis prevention strategy aimed at modulating the deleterious host inflammatory response to severe infection.

Aspirin is one of the most widely used drugs in the world today, shaping the face of modern health with its potent preventive activity against atherothrombotic vascular disease.[6] Low-doses of aspirin of <150mg/day are sufficient for the anti-platelet effect required for stroke and cardio-protection. These low-doses of aspirin are now also being recognised as mediating anti-inflammatory effects, and therefore, may be effective in preventing severe manifestations of sepsis.[7]

The cause of death in elderly patients with sepsis is usually multifactorial. Crucially though, sepsis constitutes a triggering and contributory factor for mortality. In this regard, considering multiple causes of death gives a more appropriate assessment of contributing factors to death in the elderly rather than the assessment of a single cause of death. Adding a simple preventive therapy with overlapping effects in reduction of cardiovascular and sepsis risks would be of major benefit to the health of Australia's ageing population by reducing health costs. Aspirin has the potential to fulfil that role.

Examination of the biological bases of aspirin in limiting the deleterious effects of the septic inflammatory cascade is illuminating. Aspirin has effects on at least three sepsis / inflammation pathways.

Tumour necrosis factor (TNF) and interleukin-6 are canonical pro-inflammatory cytokines. In sepsis, immune cell receptors recognise pathogen associated molecular patterns mediating intracellular signalling events that result in nuclear factor kappa – beta (NFkappaB) activation. This then results in transcription of TNF. NFkappaB activation is inhibited by aspirin and other non-steroidal anti-inflammatory drugs (NSAID's). This is mediated by inhibition of Ikk-B.[8] The concentration of NSAID's required for this inhibition has been measured to be lower than aspirin.[9] The low range of aspirin for this effect has not been defined.

Lipid mediators of sepsis have recently been described.[10] A number of these molecules act not only as anti-inflammatories but also to restore homeostasis.[11 12] In sepsis they reduce established inflammation by mechanisms including restoration of polymorphonuclear apoptosis which limits continued production of pro inflammatory cytokines in tissues and increase of nitric oxide synthesis.[13] Low-doses of aspirin have been shown to increase lipoxins (aspirin triggered lipoxin (ATL)) and resolvins in vitro [11 12] and in human trials.[14] Furthermore, ATL mediated, salutary effects have been shown in animal and human disease models of sepsis and inflammation. Animal models demonstrate that aspirin given both before and after onset of sepsis reduce mortality.[15] Low-dose aspirin increases nitric oxide production as seen in experimental animals.[16] Additionally, in a human model, ATL accounts for prevention of skin blister via reduced neutrophil and macrophage accumulation.[17] Crucially, these beneficial effects are unique to aspirin among the NSAIDs as it alone increases ATL.

Platelets become activated in sepsis due to interaction with invading bacteria via three broad mechanisms. These constitute; (i) binding of bacteria to plasma proteins which are ligands for platelet receptors, (ii) direct bacterial binding to platelet receptors and (iii) secretion of aspirin binding bacterial products such as toxins.[18] Aspirin reduces activation of platelets by inhibition of cyclooxygenase I.[19]

Bleeding risks in patients taking regular aspirin are clearly defined. Among women taking between 700 and 1625 mg aspirin per week the increased risk of all gastrointestinal haemorrhage was 1.27 (95% confidence interval 1.05-1.55).[20 21] This must be balanced against potential benefits of low-dose aspirin therapy, including for sepsis prevention.

Numerous observational studies have investigated the influence of long-term aspirin use prior to onset of sepsis.[22-34] A number of these propensity-matched studies show that long-term aspirin use is associated with reduced mortality.[22-28 30-32]

These observational data along with the effect of low-dose aspirin on inflammatory pathways provide a compelling background for investigation of possible reduction in sepsis deaths in ANTISEPSIS.

STUDY METHODS

ANTISEPSIS is a sub-study of the ASPirin in Reducing Events in the Elderly study (ASPREE). ASPREE is an Australian/US randomised, double blind, placebo controlled, primary prevention trial of low-dose aspirin in the elderly. ASPREE will measure the effect of low-dose aspirin on numerous outcomes: all-cause mortality, incident dementia, persistent physical disability, cardiovascular and major haemorrhagic events, and cancer incidence. ANTISEPSIS is embedded in the study design of the ASPREE principal study. Monitoring and event reporting for ANTISEPSIS is conducted with NHMRC funding of staff within the ASPREE data and document collection team. ANTISEPSIS is being conducted between 2012 and 2018.

HYPOTHESIS AND AIMS

We hypothesise that severe outcomes relating to sepsis in the elderly may be prevented by daily low-dose aspirin. We will conduct the ANTISEPSIS trial utilising the infrastructure of the ASPREE randomised controlled trial (RCT). We will extend sepsis event data collection in ASPREE participants to assess our primary endpoint:

- Reduction of deaths contributed to by sepsis in participants receiving aspirin versus placebo.

We will also conduct an analysis of two secondary endpoints:

- Reduction of severe infection episodes requiring hospital admissions
- Reduction of ICU admissions among patients hospitalised for severe sepsis

ANTISEPSIS STUDY DESIGN

Detailed methods for the ASPREE 'principal' study are described in the ASPREE protocol available on the aspree.org website - http://www.aspree.org/AUS/aspree-content/aspree-study- details/aspree-materials.aspx/ and in the ASPREE methods paper.[35]

Only the Australian ASPREE participants are included in the ANTISEPSIS study. This stems from the adequacy of the Australian ASPREE sample size for the ANTISEPSIS endpoints. Furthermore, the absence of detailed ICU data on participants from the United States of America means there would be insufficient data to adjudicate all sepsis endpoints.

The design of the ANTISEPSIS trial mirrors that of the principal ASPREE study. ANTISEPSIS is also a trial of 100 mg enteric-coated aspirin taken daily versus matching placebo. ASPREE trial participants were recruited through their primary care general practices. Randomisation of Australian participants was stratified for general practice and for age (70-79 or ≥ 80 years).

ANTISEPSIS recruits from ASPREE participants and hence the exclusion/inclusion criteria for the ASPREE study apply. The participants in the ASPREE principal trial were required to be free of previous cardiovascular disease or stroke, have preserved intellectual function and have no known illness that would preclude their follow up participation within the next 3 to 5 years. Apart from this they are broadly representative of the healthy elderly population.

The details on: study medication and supplies, screening visit and run-in placebo for the ASPREE trial, randomization visit, scheduled visits and measurements are as per the ASPREE protocol.[35]

ANTISEPSIS ENDPOINT DETERMINATION

Demographic data describing age, sex, body habitus, comorbidities, smoking history, alcohol intake and concomitant medications at ASPREE entry are available from the ASPREE study. Data related to the three endpoints of the ANTISEPSIS trial are available from General Practitioner (GP) clinics, hospital documentation and death certificates. Additional demographic, severity of illness and outcome data on patients admitted to Intensive Care Units (ICUs), will be obtained by merging ASPREE data with information from the Australian and New Zealand Intensive Care Society (ANZICS) Adult Patient Database (APD), one of four clinical quality registries run by the ANZICS Centre for Outcome and Resource Evaluation (CORE).

As ASPREE data only provides ICD-10 hospital primary admission codes, ANTISEPSIS will examine all hospital discharge summaries and death certificates from ASPREE participants identified as having a possible sepsis endpoint to ensure complete capture of sepsis episodes. The search for hospitalisation data relies on a number of source materials. The ASPREE operational process captures all hospitalisations although some hospital discharge summaries may be blank. In that instance GP records will be examined for information regarding the reason for hospitalisation. ASPREE participants also report admission(s) on yearly review and ASPREE operational processes also identify participant deaths. Death Certificates are retrieved for ASPREE patients who die outside of an acute hospital admission. Missing data on hospitalisation admission / discharge diagnoses are flagged for priority retrieval by ASPREE monitors / study personnel at GP visits.

ANTISEPSIS study research staff extract information relating to:

- Nature of sepsis episode, diagnosis, infecting organism (if available).

- Sepsis severity; ICU admission, disease severity at ICU admission (Acute Physiology Chronic Health Evaluation [APACHE] II score), prognostic score (APACHE risk of death [APACHE II score with adjustment for site of infection])
- Sepsis episode outcome; survival or death.
- Time of sepsis episode from ASPREE study medication commencement for survival analysis.

Data extracted from the ANZICS APD includes:

- Sepsis severity and criteria, ICU length of stay, disease severity at ICU admission (APACHE II and III scores), predicted risk of death (derived from the APACHE III scoring system and using the "ANZ Risk of Death" model), ICU and hospital outcomes.

An electronic case report form has been designed for input of ANTISEPSIS Study endpoint data into the ASPREE web-based data acquisition system.

ANTISEPSIS CASE DEFINITIONS

SEPSIS DEFINITION

Sepsis is defined as the presence of the systemic inflammatory response syndrome (SIRS; the presence of two or more of: Temperature < 36° C or > 38° C, Heart Rate > 90 beats per minute, Respiratory Rate > 20 breaths/min or PaCO2 < 32 mmHg, and White Blood Cell Count > 12,000 or < 4,000 cells/mm3 or > 10% bands) [36] plus documented infection as described below. The use of SIRS criteria for definition of sepsis relates to the timing of ANTISEPSIS protocol development and study commencement. This predated the development of the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3).[3]

INFECTION SITE DEFINITIONS

Primary blood stream infection is defined as recognised pathogen (defined as a microorganism not usually regarded as a common skin contaminant) cultured from one or more blood cultures, or a common skin contaminant cultured from two or more blood cultures drawn on separate occasions and the organism cultured from blood is not related to an infection at another site, including intravascular-access devices.[37]

Pneumonia is defined as chest radiograph within 24 hours of hospital admission demonstrating features consistent with acute pneumonia; and at least 2 symptoms consistent with pneumonia (e.g., fever or hypothermia, rigors, sweats, new cough [with or without sputum], chest discomfort, or new-onset of dyspnoea).[38]

Hospital acquired pneumonia is defined as pneumonia that occurs 48 hours or more after admission, which was not incubating at the time of admission.[39]

Meningitis is defined as increased white cells, elevated protein, and decreased glucose in CSF (per reporting laboratory's reference range), or, organisms seen on Gram stain of CSF, or organisms identified from blood by a culture or non-culture based microbiologic testing method which is performed for purposes of clinical diagnosis or treatment.[40]

Urinary tract infection [UTI] is defined as, at least one of; fever > 38^{o}C, suprapubic tenderness, costovertebral angle pain or tenderness, urinary frequency, urinary urgency or dysuria and urine culture with no more than two species of organisms at least one of which is a bacterium of ≥ 10^5 colony forming units/ml.[41] UTI is either non-catheter associated or, where a urinary catheter has bee in situ for at least two days, catheter associated.

Skin and Soft Tissue Infection

a) Cellulitis is defined as any spreading infection involving the dermis and subcutaneous tissues.[42]

b) Abscess is defined as any collection of pus within the dermis or subcutaneous tissues. [42]

c) Necrotising soft tissue infections is defined as a necrotizing infection involving any of the soft tissue layers, including the dermis, subcutaneous tissue, superficial or deep fascia, and muscle.[42]

Peritonitis is defined as a clinically compatible illness with abdominal pain and guarding associated with documented evidence of perforation (free air in the abdomen on radiographic studies or surgical confirmation of peritoneal inflammation following luminal perforation).[37]

Bone and joint infection is defined as the presence of organisms grown from bone or joint tissues by microbiologic culture in a patient with bony pain, tenderness or drainage.[40]

Infective endocarditis is diagnosed according to modified Dukes Criteria.[43]

Gastroenteritis is defined as the acute onset of diarrhoea (liquid stools for > 12 hours) and no likely non-infectious cause with an enteric pathogen identified from stool or rectal swab by a culture or non-culture based microbiologic method.[40]

The approval by the Monash University Human Research Ethics Committee for ANTISEPSIS includes authorisation to contact local pathology services to extract individual patient pathology results to confirm the presence of sepsis.

ENDPOINT DEFINITIONS

The primary endpoint, death due to sepsis, is defined as death of an ASPREE participant who had been either admitted to hospital for an infection episode or where such an episode of infection develops in hospital and in either case, the infection episode contributes to death (as determined by hospital records and/or death certificate). If ASPREE participants die out of hospital, the death certificate will be used to determine whether sepsis was a contributory cause of death.

Secondary study endpoints are defined as i) non-fatal sepsis during hospital admission of an ASPREE participant due to an infection episode defined as above, ii) non-fatal sepsis / septic shock during ICU admission of an ASPREE participant due to an infection episode defined as above.

ENDPOINT ADJUDICATION

An endpoint adjudication committee consisting of ANTISEPSIS investigators who are Infectious Diseases and ICU staff specialist physicians decides outcomes. The adjudication process is web-based and all those involved are blinded to group allocation. Two ANTISEPSIS adjudicators review each event independently. Where there is agreement this is the outcome of the event. Discordant results go to a third reviewer for the final adjudication.

TRIAL SAFE CONDUCT - DATA SAFETY MONITORING BOARD

The safety routine for ANTISEPSIS is as established for ASPREE. All serious adverse events and adverse events are registered with the ASPREE trial according to the established protocol and then presented to an independent Data Safety and Monitoring Board (DSMB; established by the National Institute on Aging), and to Human Research Ethics Committees.

The DSMB oversees the ASPREE study to monitor quality control of the data, progress of recruitment and safety aspects of the ASPREE trial.

SAMPLE SIZE AND STUDY POWER

At the time that ANTISEPSIS was designed, available observational data showed a relative effect size on sepsis mortality in ICU patients taking long-term aspirin prior to ICU admission, in the order of 40 to 80%.[22 25] These data are from two studies with fundamentally different design. One was an observational study of Australian ICU patients, which showed that patients taking aspirin prior to hospitalisation with SIRS who were continued on aspirin had an odds ratio of 0.6 for in-hospital mortality compared with those not treated with aspirin. The hazard ratio calculated from this study for mortality in SIRS patients treated with aspirin was 0.63. The group of patients with proven sepsis had an odds ratio of 0.52 for mortality.[22] The other single centre, German study of aspirin in ICU patients showed reduced mortality (OR 0.19) in patients with an unrestricted range of admission diagnoses.[25]

Using a hazard ratio of 0.63 to examine the sample size required to power our ANTISEPSIS study for the outcome of sepsis-related mortality is a conservative approach given the lower odds ratios available from the German study and Australian patients with proven sepsis. However, we reasoned that the SIRS odds ratio was the most reliable representation of the likely magnitude of aspirin effects on the inflammatory pathways common to SIRS and sepsis given that this figure is based on a large cohort (n=5525) of first SIRS episodes.

The ASPREE study rate of death was estimated to be 17.6 per 1000 participant years. Approximately 4.75 years average follow-up time per participant will be available in an analysis of time to death. We assumed that 20% of the deaths in ASPREE participants would be contributed to by sepsis (as is supported by Australian data [30]). This provides an event

rate for the primary endpoint of ANTISEPSIS of 3.5 per 1000 participant years. Anticipating half of 16,000 Australian ASPREE participants to be randomised to placebo and an event rate of 3.5 per 1000 participant year, we expect 133 primary endpoint events in the placebo group. With this number of sepsis-related deaths, we would have 80% power to detect a hazard ratio of 0.69 for aspirin versus placebo groups. If only 15% of ASPREE deaths are contributed to by sepsis, we would instead expect 100 primary end point events in the control group and still have 80% power to detect a hazard ratio of 0.65. In either of these scenarios, the ANTISEPSIS study is powered to detect an effect less than the hazard ratio of 0.63 measured previously.[26] The two secondary endpoints, admission to hospital for sepsis and admission to ICU for sepsis are more common than death due to sepsis,[3, 4] so they will be powered to detect smaller effects than outlined above for the primary endpoint.

DATA ANALYSIS

We will analyse our primary and two secondary end-points without Bonferroni correction using univariate survival analysis methods; the log-rank test and Cox proportional hazards regression. The proportional hazards assumption will be tested as part of the analysis. In subsequent analysis we will adjust for the following variables known to influence mortality due to sepsis; diabetes, age at recruitment, malignancy, alcohol intake and smoking or chronic lung disease.

The main analyses will all compare randomised groups, i.e. be intention to treat. A per protocol analysis will also be performed.

ANTISEPSIS trial results will be reported according to the CONSORT statement.[44]

ETHICAL CONDUCT OF THE TRIAL

This study will be conducted in accordance with the ICH GCP Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) annotated with TGA comments and NH&MRC National Statement on Ethical Conduct in Human Research 2007 protocols, and in keeping with local regulations.

Monash University Human Research Ethics Committee approved the ANTISEPSIS study (CF13/466 – 2013000204, 9 April 2013).

INFORMED CONSENT

The need for individual informed consent for inclusion of ASPREE participants in ANTISEPSIS has been waived by the Monash University Human Research Ethics Committee in its approval of the ANTISEPSIS study. The ANTISEPSIS study conforms to the conditions set out in CHAPTER 2.3: QUALIFYING OR WAIVING CONDITIONS FOR CONSENT in the National Statement on Ethical Conduct in Human Research 2007.

TRIAL REGISTRATION

ANTISEPSIS is registered with the Australian New Zealand Clinical Trials Registry ACTRN12613000349741. The ASPREE study is registered with the International Standard Randomised Controlled Trials Register, ASPirin in Reducing Events in the Elderly, ISRCTN83772183 and clinicaltrials.gov NCT01038583.

STUDY FUNDING

The ANTISEPSIS Study is funded by The National Health & Medical Research Council project grant #1041986 (2013-2017).

DISCUSSION

Primary prevention with aspirin appears to reduce all-cause mortality. A meta analysis of 100,000 aspirin primary prevention trial patient outcomes showed that this reduced all cause mortality (OR, 0.94; 95% CI, 0.88-1.00) and that this reduction was due neither to reduced cardiovascular or cancer deaths.[21] However, deaths due to cancer do appear to be reduced in a meta-analysis of long term follow up of 25,570 participants from primary and secondary prevention aspirin trials. Here the reduction of cancer deaths with aspirin therapy increased with duration of treatment.[45] This finding can therefore be viewed as being separate from the non-cancer reduction of all cause mortality from primary prevention studies. No analysis of non-cardiovascular or non-cancer mortality has been undertaken on data from primary or secondary aspirin prevention studies. It may be that the one of the previously unmeasured pathways by which aspirin reduces all-cause mortality is via a reduction in sepsis deaths when aspirin is used for primary prevention. We will test this hypothesis in the ANTISEPSIS trial.

Recruitment to ASPREE was completed in December 2014 with a final number of 16,703 participants in Australia. The collection of information on endpoints will conclude at the end of 2017 and the unblinding of the principal ASPREE trial, and therefore ANTISEPSIS, is expected in mid-2018. If aspirin is shown to be effective in reducing the impact of severe sepsis, it will be the first time that such an outcome has been demonstrated.

ACNOWLEDGEMENTS

The investigators acknowledge the National Institute on Aging (NIA; #RO1-AG029824) for funding the principal ASPREE trial in Australia and the USA, and the National Health & Medical Research Council (grant #334047), the Victorian Cancer Agency and Monash University for funding support of ASPREE in Australia. The investigators also acknowledge the work of all ASPREE field research staff, and those within the ASPREE data team who source the supporting documents from hospitals and pathology services. The investigators would particularly like to acknowledge the valued contribution of the ASPREE participants and the support from their general practitioners.

AUTHORS' CONTRIBUTIONS.

DPE conceived the study.

DPE, KL, ESMcB, DP, RW and RLW wrote the study protocol.

JL designed the database used in the study.

JJM is responsible for the ASPREE study on which this study protocol is based.

ESMcB and RLW designed the statistical analysis plan fort he protocol.

DPE, KL, ESMcB, DP, RW and EM designed the implementation aspects of this protocol.

All authors critically reviewed the manuscript.

COMPETING INTERESTS STATEMENT.

None of the authors have competing interests.

REFERENCES

1. World Health Organisation. The top 10 causes of death. 2014. http://www.who.int/mediacentre/factsheets/fs310/en/index.html.

2. Global Burden of Diseases Study, Mortality, Causes of Death Committee. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 2015;**385**(9963):117-71 doi: 10.1016/S0140-6736(14)61682-2.

3. Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA 2016;**315**(8):801-10 doi: 10.1001/jama.2016.0287.

4. Kaukonen KM, Bailey M, Suzuki S, Pilcher D, Bellomo R. Mortality related to severe sepsis and septic shock among critically ill patients in Australia and New Zealand, 2000-2012. JAMA 2014;**311**(13):1308-16 doi: 10.1001/jama.2014.2637.

5. Cohen J, Vincent JL, Adhikari NK, et al. Sepsis: a roadmap for future research. Lancet Infect Dis 2015;**15**(5):581-614 doi: 10.1016/S1473-3099(15)70112-X.

6. Goodman SG, Menon V, Cannon CP, Steg G, Ohman EM, Harrington RA. Acute ST-segment elevation myocardial infarction: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). Chest 2008;**133**(6 Suppl):708S-75S

7. Eisen DP. Manifold beneficial effects of acetyl salicylic acid and nonsteroidal anti-inflammatory drugs on sepsis. Intensive Care Med 2012;**38**(8):1249-57 doi: 10.1007/s00134-012-2570-8.

8. Kopp E, Ghosh S. Inhibition of NF-kappa B by sodium salicylate and aspirin. Science 1994;**265**(5174):956-9

9. Takada Y, Bhardwaj A, Potdar P, Aggarwal BB. Nonsteroidal anti-inflammatory agents differ in their ability to suppress NF-kappaB activation, inhibition of expression of cyclooxygenase-2 and cyclin D1, and abrogation of tumor cell proliferation. Oncogene 2004;**23**(57):9247-58

10. Serhan CN, Haeggstrom JZ, Leslie CC. Lipid mediator networks in cell signaling: update and impact of cytokines. FASEB J 1996;**10**(10):1147-58

11. Serhan CN, Chiang N. Lipid-derived mediators in endogenous anti-inflammation and resolution: lipoxins and aspirin-triggered 15-epi-lipoxins. Scientific World Journal 2002;**2**:169-204 doi: 10.1100/tsw.2002.81.

12. Arita M, Yoshida M, Hong S, et al. Resolvin E1, an endogenous lipid mediator derived from omega-3 eicosapentaenoic acid, protects against 2,4,6-trinitrobenzene sulfonic acid-induced colitis. Proc Natl Acad Sci USA 2005;**102**(21):7671-6 doi: 10.1073/pnas.0409271102.

13. Paul-Clark MJ, Van Cao T, Moradi-Bidhendi N, Cooper D, Gilroy DW. 15-epi-lipoxin A4-mediated induction of nitric oxide explains how aspirin inhibits acute inflammation. J Exp Med 2004;**200**(1):69-78 doi: 10.1084/jem.20040566.

14. Chiang N, Bermudez EA, Ridker PM, Hurwitz S, Serhan CN. Aspirin triggers antiinflammatory 15-epi-lipoxin A4 and inhibits thromboxane in a randomized human trial. Proc Natl Acad Sci USA 2004;**101**(42):15178-83 doi: 10.1073/pnas.0405445101.

15. El Kebir D, Jozsef L, Pan W, et al. 15-epi-lipoxin A4 inhibits myeloperoxidase signaling and enhances resolution of acute lung injury. Am J Respir Crit Care Med 2009;**180**(4):311-9 doi: 10.1164/rccm.200810-1601OC.

16. Hennekens CH, Schneider WR, Pokov A, et al. A randomized trial of aspirin at clinically relevant doses and nitric oxide formation in humans. J Cardiovasc Pharmacol Ther 2010;**15**(4):344-8 doi: 10.1177/1074248410375091.

17. Morris T, Stables M, Hobbs A, et al. Effects of low-dose aspirin on acute inflammatory responses in humans. J Immunol 2009;**183**(3):2089-96 doi: jimmunol.0900477 [pii] 10.4049/jimmunol.0900477.

18. Cox D, Kerrigan SW, Watson SP. Platelets and the innate immune system: mechanisms of bacterial-induced platelet activation. J Thromb Haemost 2011;**9**(6):1097-107 doi: 10.1111/j.1538-7836.2011.04264.x.

19. Masotti G, Galanti G, Poggesi L, Abbate R, Neri Serneri GG. Differential inhibition of prostacyclin production and platelet aggregation by aspirin in humans. Adv Prostaglandin Thromboxane Res 1980;**6**:317-20

20. Huang ES, Strate LL, Ho WW, Lee SS, Chan AT. Long-term use of aspirin and the risk of gastrointestinal bleeding. Am J Med 2011;**124**(5):426-33 doi: 10.1016/j.amjmed.2010.12.022.

21. Seshasai SR, Wijesuriya S, Sivakumaran R, et al. Effect of aspirin on vascular and nonvascular outcomes: meta-analysis of randomized controlled trials. Arch Intern Med 2012;**172**(3):209-16 doi: 10.1001/archinternmed.2011.628.

22. Eisen DP, Reid D, McBryde ES. Acetyl salicylic acid usage and mortality in critically ill patients with the systemic inflammatory response syndrome and sepsis. Crit Care Med 2012;**40**(6):1761-7 doi: 10.1097/CCM.0b013e318246b9df.

23. Lösche W BJ, Kabisch B, Winning J, Claus RA, Bauer M. . Do aspirin and other antiplatelet drugs reduce the mortality in critically ill patients? Thrombosis 2011(2012)

24. Winning J, Neumann J, Kohl M, et al. Antiplatelet drugs and outcome in mixed admissions to an intensive care unit. Crit Care Med 2010;**38**(1):32-7 doi: 10.1097/CCM.0b013e3181b4275c.

25. Winning J, Reichel J, Eisenhut Y, et al. Anti-platelet drugs and outcome in severe infection: clinical impact and underlying mechanisms. Platelets 2009;**20**(1):50-7 doi: 908188675 [pii] 10.1080/09537100802503368.

26. Chen W JD, Bastarache JA, May AK, O'Neal Jr HR, Bernard GR, et al. Prehospital aspirin use is associated with reduced risk of acute respiratory distress syndrome in critically ill patients: a propensity-adjusted analysis. Crit Care Med 2015;**43**(4):801-07

27. Otto GP SM, Boettel J, Kabisch B, Breuel H, Winning J, et al. Effects of low-dose acetylsalicylic acid and atherosclerotic vascular diseases on the outcome in patients with severe sepsis or septic shock. Platelets 2013;**24**(6):480-85

28. Valerio-Rojas JC, Jaffer IJ, Kor DJ, Gajic O, Cartin-Ceba R. Outcomes of severe sepsis and septic shock patients on chronic antiplatelet treatment: a historical cohort study. Crit Care Res Pract 2013;**2013**:782573 doi: 10.1155/2013/782573.

29. Boyle AJ, Di Gangi S, Hamid UI, et al. Aspirin therapy in patients with acute respiratory distress syndrome (ARDS) is associated with reduced intensive care unit mortality: a prospective analysis. Crit Care 2015;**19**:109 doi: 10.1186/s13054-015-0846-4.

30. Falcone M RA, Cangemi R, Farcomeni A, Calvieri C, Barillà F, et al. . Lower mortality rate in elderly patients with community-onset pneumonia on treatment with aspirin. J Am Heart Assoc 2015;**4**(1):e001595

31. Sossdorf M OG, Boettel J, Winning J, Lösche W. Benefit of low-dose aspirin and non-steroidal anti-inflammatory drugs in septic patients. Crit Care 2013;**17**(1):1-2

32. Tsai MJ, Ou SM, Shih CJ, et al. Association of prior antiplatelet agents with mortality in sepsis patients: a nationwide population-based cohort study. Intensive Care Med 2015;**41**(5):806-13 doi: 10.1007/s00134-015-3760-y.

33. Al Harbi SA, Tamim HM, Al-Dorzi HM, Sadat M, Arabi YM. Association between aspirin therapy and the outcome in critically ill patients: a nested cohort study. BMC Pharmacol Toxicol 2016;**17**:5 doi: 10.1186/s40360-016-0047-z.

34. Wiewel MA, de Stoppelaar SF, van Vught LA, et al. Chronic antiplatelet therapy is not associated with alterations in the presentation, outcome, or host response biomarkers during sepsis: a propensity-matched analysis. Intensive Care Med 2016;**42**(3):352-60 doi: 10.1007/s00134-015-4171-9.

35. ASPREE Investigators Group. Study design of ASPirin in Reducing Events in the Elderly (ASPREE): a randomized, controlled trial. Contemp Clin Trials 2013;**36**(2):555-64 doi: 10.1016/j.cct.2013.09.014.

36. Bone RC, Balk RA, Cerra FB, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. Chest 1992;**101**(6):1644-55

37. Calandra T, Cohen J, International Sepsis Forum Definition of Infection in the ICUCC. The international sepsis forum consensus conference on definitions of infection in the intensive care unit. Crit Care Med 2005;**33**(7):1538-48

38. Mandell LA, Wunderink RG, Anzueto A, et al. Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. Clin Infect Dis 2007;**44 Suppl 2**:S27-72 doi: 10.1086/511159.

39. Tablan OC, Anderson LJ, Besser R, et al. Guidelines for preventing health-care--associated pneumonia, 2003: recommendations of CDC and the Healthcare Infection Control Practices Advisory Committee. MMWR Recomm Rep 2004;**53**(RR-3):1-36

40. Centers for Disease Control. CDC/NHSN Surveillance Definitions for Specific Types of Infections. Secondary CDC/NHSN Surveillance Definitions for Specific Types of Infections 2016. http://www.cdc.gov/nhsn/PDFs/pscManual/17pscNosInfDef_current.pdf.

41. Centers for Disease Control. Urinary Tract Infection (Catheter-Associated Urinary Tract Infection [CAUTI] and Non-Catheter-Associated Urinary Tract Infection [UTI]) and Other Urinary System Infection [USI]) Events. Secondary Urinary Tract Infection (Catheter-Associated Urinary Tract Infection [CAUTI] and Non-Catheter-Associated Urinary Tract Infection [UTI]) and Other Urinary System Infection [USI]) Events 2016. http://www.cdc.gov/nhsn/PDFs/pscManual/17pscNosInfDef_current.pdf.

42. Gunderson CG. Cellulitis: definition, etiology, and clinical features. Am J Med 2011;**124**(12):1113-22 doi: 10.1016/j.amjmed.2011.06.028.

43. Li JS, Sexton DJ, Mick N, et al. Proposed modifications to the Duke criteria for the diagnosis of infective endocarditis. Clin Infect Dis 2000;**30**(4):633-8 doi: 10.1086/313753.

44. Moher D, Hopewell S, Schulz KF, et al. CONSORT 2010 Explanation and Elaboration: Updated guidelines for reporting parallel group randomised trials. J Clin Epidemiol 2010;**63**(8):e1-37 doi: 10.1016/j.jclinepi.2010.03.004.

45. Rothwell PM, Fowkes FG, Belch JF, Ogawa H, Warlow CP, Meade TW. Effect of daily aspirin on long-term risk of death due to cancer: analysis of individual patient data

from randomised trials. Lancet 2011;**377**(9759):31-41 doi: 10.1016/S0140-6736(10)62110-1.

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	3
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	5
	2b	Specific objectives or hypotheses	9
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	10
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	N/A
Participants	4a	Eligibility criteria for participants	10
	4b	Settings and locations where the data were collected	10
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	10 9
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	N/A
	6b	Any changes to trial outcomes after the trial commenced, with reasons	16
Sample size	7a	How sample size was determined	N/A
	7b	When applicable, explanation of any interim analyses and stopping guidelines	
Randomisation: Sequence generation	8a	Method used to generate the random allocation sequence	10 reference to the principle study protocol

			http://www.aspree.org/AUS/aspree-content/aspree-study-details/aspree-materials.aspx/
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	10
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	10
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	10
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	N/A
	11b	If relevant, description of the similarity of interventions	
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	17
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	N/A
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	P 3 8350
	13b	For each group, losses and exclusions after randomisation, together with reasons	10
Recruitment	14a	Dates defining the periods of recruitment and follow-up	3
	14b	Why the trial ended or was stopped	N/a
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	N/A
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	N/A
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	N/A
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	N/A

Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	N/A
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	N/A
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	4
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	N/A
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	N/A
Other information			
Registration	23	Registration number and name of trial registry	3
Protocol	24	Where the full trial protocol can be accessed, if available	N/A
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	20

NB: N/A Not applicable as this is a protocol paper.

BMJ Open

The AspiriN To Inhibit SEPSIS (ANTISEPSIS) randomised controlled trial protocol.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2016-013636.R1
Article Type:	Protocol
Date Submitted by the Author:	25-Nov-2016
Complete List of Authors:	eisen, damon; James Cook University Division of Tropical Health and Medicine, Moore, Elizabeth; Monash University, Epidemiology and Preventive Medicine leder, karin; Monash University, Epidemiology and Preventive Medicine Lockery, Jessica; Monash University, Epidemiology and Preventive Medicine mcbryde, emma; James Cook University Division of Tropical Health and Medicine, Australian Institute of Tropical Health and Medicine McNeil, John; Monash University, Epidemiology & Preventive Medicine Pilcher, David; Australian and New Zealand Intensive Care Society Centre for Outcomes and Resource Evaluation, Melbourne, VIC, Australia Wolf, Rory; Monash University, Epidemiology and Preventive Medicine woods, robyn; Monash University, Epidemiology and Preventive Medicine
Primary Subject Heading:	Public health
Secondary Subject Heading:	Infectious diseases, Intensive care
Keywords:	Public health < INFECTIOUS DISEASES, Adult intensive & critical care < INTENSIVE & CRITICAL CARE, Microbiology < BASIC SCIENCES

The AspiriN To Inhibit SEPSIS (ANTISEPSIS) randomised controlled trial protocol.

Damon P Eisen 1,2,3#

Elizabeth M Moore 3

Karin Leder 3,4

Jessica Lockery 3

Emma S McBryde 5#

John J McNeil 3

Dave Pilcher 3,6,7

Rory Wolfe 3

Robyn L Woods 3

1. Townsville Hospital and Health Service

   100 Angus Smith Drive, Douglas QLD 4814 AUSTRALIA

2. College of Medicine and Dentistry

Division of Tropical Health and Medicine

James Cook University Townsville QLD 4811 AUSTRALIA

3. Department of Epidemiology and Preventive Medicine

Monash University, Commercial Rd, Prahran, Victoria, 3184 AUSTRALIA

4. Victorian Infectious Diseases Service at the Peter Doherty Institute

Royal Melbourne Hospital, 792 Elizabeth St, Melbourne, Victoria, 3000 AUSTRALIA

5. Australian Institute of Tropical Health and Medicine

   Division of Tropical Health and Medicine

James Cook University Townsville QLD 4811 AUSTRALIA

6. Department of Intensive Care, The Alfred Hospital, Commercial Road, Prahran, Victoria 3004 AUSTRALIA
7. The Australian and New Zealand Intensive Care Society (ANZICS) Centre for Outcome and Resource Evaluation (CORE), Ievers Terrace, Carlton, Victoria 3154 AUSTRALIA

Formerly affiliated with Victorian Infectious Diseases Service at the Peter Doherty Institute, Royal Melbourne Hospital

Protocol Version 1.0 12.11.16

Corresponding author; Damon Eisen

Townsville Hospital and Health Service

100 Angus Smith Drive, Douglas QLD 4814 AUSTRALIA

Telephone: +61 7 4433 2459

Fax: +61 7 4433 1271

Email. damon.eisen@health.qld.gov.au

Keywords: Aspirin, primary prevention, elderly, sepsis, death, hospitalisation.

Word Count inc. World Health Organization Trial Registration Data Set

: 4568

Word Count Main text: 4018

World Health Organization Trial Registration Data Set

Primary Registry and Trial Identifying Number

ANTISEPSIS is registered with the Australian New Zealand Clinical Trials Registry ACTRN12613000349741.

Date of Registration in Primary Registry

02/04/2013

Secondary Identifying Numbers

The ASPREE study is registered with the International Standard Randomised Controlled Trials Register, ASPirin in Reducing Events in the Elderly, ISRCTN83772183 and clinicaltrials.gov NCT01038583.

Source(s) of Monetary or Material Support

The ANTISEPSIS Study is funded by The National Health & Medical Research Council project grant #1041986 (2013-2017).

Primary Sponsor

Monash University, Clayton, Victoria, 3800, Australia

Secondary Sponsor(s)

Not applicable

Contact for Public and Scientific Queries

Damon Eisen

Townsville Hospital and Health Service

100 Angus Smith Drive, Douglas QLD 4814 AUSTRALIA

Telephone: +61 7 4433 2459

Fax: +61 7 4433 1271

Email. damon.eisen@health.qld.gov.au

Public Title

Aspirin to prevent death associated with sepsis

Scientific Title

AspiriN To Inhibit SEPSIS (ANTISEPSIS) PROTOCOL – AN ASPREE SUBSTUDY

Countries of Recruitment

Australia, USA

Health Condition(s) or Problem(s) Studied

Healthy human volunteer cohort. Prevention of death due to sepsis (evidence of infection plus the systemic inflammatory response syndrome).

Intervention(s)

Daily aspirin 100mg versus matching placebo.

Key Inclusion and Exclusion Criteria

Inclusion criteria;

- 70 years of age or older
- free of previous cardiovascular disease or stroke
- preserved intellectual function

Exclusion criteria;

- known illness that would preclude participation for three to five years.

Study Type

Primary prevention, double blind, randomised, placebo controlled trial.

The randomization list was generated by an independent statistician ensuring that the randomization code remained inaccessible to all study staff and senior investigators. The

randomization list was generated using the STATA 'ralloc' procedure with randomization stratified for age (<80 yrs. and >80 yrs.). Following the completion of the randomization process by the research assistant / nurse, a study medication number will be provided. All staff remain blinded to treatment allocation through the randomization procedure.

Study documents are only marked with a unique, participant trial identity code. Study documents had patient details blanked out for endpoint adjudication by the trial's investigators.

Date of First Enrollment

10/01/2011

Target Sample Size

16,500

Recruitment Status

Recruiting.

Primary Outcome(s)

Sepsis is defined as proven infection plus at least two of the four Systemic Inflammatory Response Syndrome criteria. (Temperature < 36° C or > 38° C, Heart Rate > 90 beats per minute, Respiratory Rate > 20 breaths/min or PaCO2 < 32 mmHg, and White Blood Cell Count > 12,000 or < 4,000 cells/mm3 or > 10% bands).

The primary endpoint, death due to sepsis, is defined as death of an ANTISEPSIS participant who had been either admitted to hospital for an infection episode or where such an episode of infection develops in hospital and in either case, the infection episode contributes to death (as determined by hospital records and/or death certificate). If ANTISEPSIS participants die out of hospital, the death certificate will be used to determine whether

sepsis was a contributory cause of death. This primary outcome will be measured during the ANTISEPSIS trial.

Key Secondary Outcomes

1. Non-fatal sepsis during hospital admission of an ANTISEPSIS participant due to an infection episode that had either led to admission to hospital or where such an episode of infection develops in hospital.
2. Non-fatal sepsis / septic shock during ICU admission of an ANTISEPSIS participant due to an infection episode that had either led to admission to hospital or where such an episode of infection develops in hospital.

ABSTRACT

Introduction. Sepsis is a leading global cause of morbidity and mortality, and is more common at the extremes of age. Moreover, the cost of in-hospital care for elderly patients with sepsis is significant. There are indications from experimental and observational studies that aspirin may reduce inflammation associated with infection. This paper describes the rationale and design of the AspiriN To Inhibit SEPSIS (ANTISEPSIS) trial, a sub-study of ASPirin in Reducing Events in the Elderly (ASPREE). ANTISEPSIS primarily aims to determine whether low-dose aspirin reduces sepsis-related deaths in older people. Additionally it will assess whether low-dose aspirin reduces sepsis-related hospitalisations and sepsis-related Intensive Care Unit (ICU) admissions.

Methods and analysis: ASPREE is a double-blinded, randomised, placebo-controlled primary prevention trial that will determine whether daily low-dose aspirin extends disability-free longevity in 19,000 healthy older people recruited in Australia and the United States (USA). The ANTISEPSIS sub-study involves additional ASPREE trial data collection to assess the impact of daily low-dose aspirin on sepsis-related events in the 16,703 ASPREE participants aged 70 years and over, recruited in Australia. The intervention is a daily 100 mg dose of enteric-coated aspirin versus matching placebo, with 1:1 randomisation. The primary outcome for the ANTISEPSIS sub-study is the incidence of sepsis-related death in eligible patients. The incidence of sepsis-related hospital and ICU admissions are secondary outcomes. ANTISEPSIS is to be conducted between 2012 and 2018.

Discussion: This sub-study will determine whether aspirin, an inexpensive and accessible therapy, safely reduces sepsis-related deaths and hospitalisations in older Australians. If shown to be the case this would have profound effects on the health of older Australians.

Trial Registration: ANTISEPSIS Australian New Zealand Clinical Trials Registry identifier:

ACTRN12613000349741.

Strengths and limitations of this study

This trial uses the unequivocal measure of death due to sepsis as its primary endpoint.

This large scale, primary prevention, trial will be adequately powered to test the study hypothesis.

There is, however, no opportunity to examine the biological basis for any demonstrated effect, as real-time samples are not available at the time of sepsis episodes.

INTRODUCTION

Joseph Lister pioneered a crucial aspect of modern health practice when he first used carbolic acid solution to prevent severe infection in a contaminated wound. Antisepsis is used in all aspects of health care and daily life to protect us from pathogenic microorganisms. With the AspiriN To Inhibit SEPSIS (ANTISEPSIS) trial, we aim to confirm whether a simple and cheap health intervention can protect against manifestations of severe infection by modulating the human host response to infection, regardless of the specific causative microbe.

Sepsis is a lethal condition that kills one person a minute globally. Lower respiratory tract infections alone, caused 5.5% of all deaths in 2012, making them the third most common cause of death.[1] More specifically, in relation to our study, lower respiratory tract infections are the cause of an extremely high number of sepsis deaths in the elderly in the developed world.[2]

Septic shock has hospital mortality in excess of 40%.[3] Incremental reduction in sepsis mortality has been achieved in high-income countries through early recognition and optimisation of immediate treatment delivered in well-resourced hospitals.[4] However, this reduction mainly parallels general improvements in mortality of patients admitted to ICU.

Sepsis therapy research is bedevilled with failed attempts at proving efficacy of specific treatments.[5] Adjuvant treatments for sepsis that have been developed and trialled at major cost have failed to deliver significant reduction in mortality. There is no effective sepsis prevention strategy aimed at modulating the deleterious host inflammatory response to severe infection.

Aspirin is one of the most widely used drugs in the world today, shaping the face of modern health with its potent preventive activity against atherothrombotic vascular disease.[6] Low-doses of aspirin of < 150 mg/day are sufficient for the anti-platelet effect required for stroke and cardio-protection. These low-doses of aspirin are now also being recognised as mediating anti-inflammatory effects, and therefore, may be effective in preventing severe manifestations of sepsis.[7]

The cause of death in elderly patients with sepsis is usually multifactorial. Crucially though, sepsis constitutes a triggering and contributory factor for mortality. In this regard, considering multiple causes of death gives a more appropriate assessment of contributing factors to death in the elderly rather than the assessment of a single cause of death. Adding a simple preventive therapy with overlapping effects in reduction of cardiovascular and sepsis risks would be of major benefit to the health of Australia's ageing population by reducing health costs. Aspirin has the potential to fulfil that role.

Examination of the biological bases of aspirin in limiting the deleterious effects of the septic inflammatory cascade is illuminating. Aspirin has effects on at least three sepsis / inflammation pathways.

Tumour necrosis factor (TNF) and interleukin-6 are canonical pro-inflammatory cytokines. In sepsis, immune cell receptors recognise pathogen associated molecular patterns mediating intracellular signalling events that result in nuclear factor kappa – beta (NFkappaB) activation. This then results in transcription of TNF. NFkappaB activation is inhibited by aspirin and other non-steroidal anti-inflammatory drugs (NSAID's). This is mediated by inhibition of Ikk-B.[8] The concentration of NSAID's required for this inhibition has been measured to be lower than aspirin.[9] The low range of aspirin for this effect has not been defined.

Lipid mediators of sepsis have recently been described.[10] A number of these molecules act not only as anti-inflammatories but also to restore homeostasis.[11 12] In sepsis they reduce established inflammation by mechanisms including restoration of polymorphonuclear apoptosis which limits continued production of pro inflammatory cytokines in tissues and increase of nitric oxide synthesis.[13] Low-doses of aspirin have been shown to increase lipoxins (aspirin triggered lipoxin (ATL)) and resolvins in vitro [11 12] and in human trials.[14] Furthermore, ATL mediated, salutary effects have been shown in animal and human disease models of sepsis and inflammation. Animal models demonstrate that aspirin given both before and after onset of sepsis reduce mortality.[15] Low-dose aspirin increases nitric oxide production as seen in experimental animals.[16] Additionally, in a human model, ATL accounts for prevention of skin blister via reduced neutrophil and macrophage accumulation.[17] Crucially, these beneficial effects are unique to aspirin among the NSAIDs as it alone increases ATL.

Platelets become activated in sepsis due to interaction with invading bacteria via three broad mechanisms. These constitute; (i) binding of bacteria to plasma proteins which are ligands for platelet receptors, (ii) direct bacterial binding to platelet receptors and (iii) secretion of aspirin binding bacterial products such as toxins.[18] Aspirin reduces activation of platelets by inhibition of cyclooxygenase I.[19]

Bleeding risks in patients taking regular aspirin are clearly defined. Among primary prevention trial participants taking between 700 and 1625 mg aspirin per week the increased risk of all gastrointestinal haemorrhage was 1.27 (95% confidence interval 1.05-1.55).[20 21] This must be balanced against potential benefits of low-dose aspirin therapy, including for sepsis prevention.

Numerous observational studies have investigated the influence of long-term aspirin use prior to onset of sepsis.[22-34] A number of these propensity-matched studies show that long-term aspirin use is associated with reduced mortality.[22-28 30-32]

These observational data along with the effect of low-dose aspirin on inflammatory pathways provide a compelling background for investigation of possible reduction in sepsis deaths in ANTISEPSIS. Therefore, this doubled blind, placebo controlled, randomised controlled primary prevention trial (RCT) will investigate whether low-dose aspirin reduces deaths due to sepsis.

STUDY METHODS

ANTISEPSIS is a sub-study of the ASPirin in Reducing Events in the Elderly study (ASPREE). ASPREE is an Australian / USA randomised, double blind, placebo controlled, primary prevention trial of low-dose aspirin in the elderly. ASPREE will measure the effect of low-dose aspirin on numerous outcomes: all-cause mortality, incident dementia, persistent physical disability, cardiovascular and major haemorrhagic events, and cancer incidence. ANTISEPSIS is embedded in the study design of the ASPREE principal study. Monitoring and event reporting for ANTISEPSIS is conducted with NHMRC funding of staff within the ASPREE data and document collection team. ANTISEPSIS is being conducted between 2012 and 2018.

HYPOTHESIS AND AIMS

We hypothesise that severe outcomes relating to sepsis in the elderly may be prevented by daily low-dose aspirin. We will conduct the ANTISEPSIS trial utilising the infrastructure of the ASPREE RCT. We will extend sepsis event data collection in ASPREE participants to assess our primary endpoint:

- Reduction of deaths contributed to by sepsis in participants receiving aspirin versus placebo.

We will also conduct an analysis of two secondary endpoints:

- Reduction of sepsis episodes requiring hospital admissions
- Reduction of Intensive Care Unit (ICU) admissions among patients hospitalised for severe sepsis

ANTISEPSIS STUDY DESIGN

Detailed methods for the ASPREE 'principal' study are described in the ASPREE protocol available on the aspree.org website - http://www.aspree.org/AUS/aspree-content/aspree-study- details/aspree-materials.aspx/ and in the ASPREE methods paper.[35]

Only the Australian ASPREE participants are included in the ANTISEPSIS study. This stems from the adequacy of the Australian ASPREE sample size for the ANTISEPSIS endpoints. Furthermore, the absence of detailed ICU data on participants from the USA means there would be insufficient data to adjudicate all sepsis endpoints.

The design of the ANTISEPSIS trial mirrors that of the principal ASPREE study. ANTISEPSIS is also a trial of 100 mg enteric-coated aspirin taken daily versus matching placebo. ASPREE trial participants were recruited through their primary care general practices. One to one randomisation of Australian participants was stratified for general practice and for age (70-79 or ≥ 80 years).

ANTISEPSIS recruits from ASPREE participants and hence the exclusion/inclusion criteria for the ASPREE study apply. The participants in the ASPREE principal trial were required to be free of previous cardiovascular disease or stroke, have preserved intellectual function and have no known illness that would preclude their follow up participation within the next 3 to 5 years. Apart from this they are broadly representative of the healthy elderly population.

The details on: study medication and supplies, screening visit and run-in placebo for the ASPREE trial, randomization visit, scheduled visits and measurements are as per the ASPREE protocol.[35]

ANTISEPSIS ENDPOINT DETERMINATION

Demographic data describing age, sex, body habitus, comorbidities, smoking history, alcohol intake and concomitant medications at ASPREE entry are available from the ASPREE study. Data related to the three endpoints of the ANTISEPSIS trial are available from General Practitioner (GP) clinics, hospital documentation and death certificates. Additional demographic, severity of illness and outcome data on patients admitted to ICUs, will be obtained by merging ASPREE data with information from the Australian and New Zealand Intensive Care Society (ANZICS) Adult Patient Database (APD), one of four clinical quality registries run by the ANZICS Centre for Outcome and Resource Evaluation (CORE).

As ASPREE data only provides ICD-10 hospital primary admission codes, ANTISEPSIS will examine all hospital discharge summaries and death certificates from ASPREE participants identified as having a possible sepsis endpoint to ensure complete capture of sepsis episodes. The search for hospitalisation data relies on a number of source materials. The ASPREE operational process captures all hospitalisations although some hospital discharge summaries may be blank. In that instance GP records will be examined for information regarding the reason for hospitalisation. ASPREE participants also report admission(s) on yearly review and ASPREE operational processes also identify participant deaths. Death Certificates are retrieved for ASPREE patients who die outside of an acute hospital admission. Missing data on hospitalisation admission / discharge diagnoses are flagged for priority retrieval by ASPREE monitors / study personnel at GP visits.

ANTISEPSIS study research staff extract information relating to:

- Nature of sepsis episode, diagnosis, infecting organism (if available).

- Sepsis severity; ICU admission, disease severity at ICU admission (Acute Physiology Chronic Health Evaluation [APACHE] II score), prognostic score (APACHE risk of death [APACHE II score with adjustment for site of infection])
- Sepsis episode outcome; survival or death.
- Time of sepsis episode from ASPREE study medication commencement for survival analysis.

Data extracted from the ANZICS APD includes:

- Sepsis severity and criteria, ICU length of stay, disease severity at ICU admission (APACHE II and III scores), predicted risk of death (derived from the APACHE III scoring system and using the "ANZ Risk of Death" model), ICU and hospital outcomes.

An electronic case report form has been designed for input of ANTISEPSIS Study endpoint data into the ASPREE web-based data acquisition system.

ANTISEPSIS CASE DEFINITIONS

SEPSIS DEFINITION

Sepsis is defined as the presence of the systemic inflammatory response syndrome (SIRS; the presence of two or more of: Temperature < 36° C or > 38° C, Heart Rate > 90 beats per minute, Respiratory Rate > 20 breaths/min or PaCO2 < 32 mmHg, and White Blood Cell Count > 12,000 or < 4,000 cells/mm3 or > 10% bands) [36] plus documented infection as described below. The use of SIRS criteria for definition of sepsis relates to the timing of ANTISEPSIS protocol development and study commencement. ANTISEPSIS commenced before the development of the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3).[3]

INFECTION SITE DEFINITIONS

Primary blood stream infection is defined as recognised pathogen (defined as a microorganism not usually regarded as a common skin contaminant) cultured from one or more blood cultures, or a common skin contaminant cultured from two or more blood cultures drawn on separate occasions and the organism cultured from blood is not related to an infection at another site, including intravascular-access devices.[37]

Pneumonia is defined as chest radiograph within 24 hours of hospital admission demonstrating features consistent with acute pneumonia; and at least 2 symptoms consistent with pneumonia (e.g., fever or hypothermia, rigors, sweats, new cough [with or without sputum], chest discomfort, or new-onset of dyspnoea).[38]

Hospital acquired pneumonia is defined as pneumonia that occurs 48 hours or more after admission, which was not incubating at the time of admission.[39]

Meningitis is defined as increased white cells, elevated protein, and decreased glucose in CSF (per reporting laboratory's reference range), or, organisms seen on Gram stain of CSF, or organisms identified from blood by a culture or non-culture based microbiologic testing method which is performed for purposes of clinical diagnosis or treatment.[40]

Urinary tract infection [UTI] is defined as, at least one of; fever > 38^{o}C, suprapubic tenderness, costovertebral angle pain or tenderness, urinary frequency, urinary urgency or dysuria and urine culture with no more than two species of organisms at least one of which is a bacterium of ≥ 10^5 colony forming units/ml.[41] UTI is either non-catheter associated or, where a urinary catheter has bee in situ for at least two days, catheter associated.

Skin and Soft Tissue Infection

a) Cellulitis is defined as any spreading infection involving the dermis and subcutaneous tissues.[42]

b) Abscess is defined as any collection of pus within the dermis or subcutaneous tissues. [42]

c) Necrotising soft tissue infections is defined as a necrotizing infection involving any of the soft tissue layers, including the dermis, subcutaneous tissue, superficial or deep fascia, and muscle.[42]

Peritonitis is defined as a clinically compatible illness with abdominal pain and guarding associated with documented evidence of perforation (free air in the abdomen on radiographic studies or surgical confirmation of peritoneal inflammation following luminal perforation).[37]

Bone and joint infection is defined as the presence of organisms grown from bone or joint tissues by microbiologic culture in a patient with bony pain, tenderness or drainage.[40]

Infective endocarditis is diagnosed according to modified Dukes Criteria.[43]

Gastroenteritis is defined as the acute onset of diarrhoea (liquid stools for > 12 hours) and no likely non-infectious cause with an enteric pathogen identified from stool or rectal swab by a culture or non-culture based microbiologic method.[40]

The approval by the Monash University Human Research Ethics Committee for ANTISEPSIS includes authorisation to contact local pathology services to extract individual patient pathology results to confirm the presence of sepsis.

ENDPOINT DEFINITIONS

The primary endpoint, death due to sepsis, is defined as death of an ASPREE participant who had been either admitted to hospital for an infection episode or where such an episode of infection develops in hospital and in either case, the infection episode contributes to death (as determined by hospital records and/or death certificate). If ASPREE participants die out of hospital, the death certificate will be used to determine whether sepsis was a contributory cause of death.

Secondary study endpoints are defined as i) non-fatal sepsis during hospital admission of an ASPREE participant due to an infection episode defined as above, ii) non-fatal sepsis / septic shock during ICU admission of an ASPREE participant due to an infection episode defined as above.

ENDPOINT ADJUDICATION

An endpoint adjudication committee consisting of ANTISEPSIS investigators who are Infectious Diseases and ICU staff specialist physicians decides outcomes. The adjudication process is web-based and all those involved are blinded to group allocation. Two ANTISEPSIS adjudicators review each event independently. Where there is agreement this is the outcome of the event. Discordant results go to a third reviewer for the final adjudication.

TRIAL SAFE CONDUCT - DATA SAFETY MONITORING BOARD

The safety routine for ANTISEPSIS is as established for ASPREE. All serious adverse events and adverse events are registered with the ASPREE trial according to the established protocol and then presented to an independent Data Safety and Monitoring Board (DSMB; established by the National Institute on Aging), and to Human Research Ethics Committees.

The DSMB oversees the ASPREE study to monitor quality control of the data, progress of recruitment and safety aspects of the ASPREE trial.

SAMPLE SIZE AND STUDY POWER

At the time that ANTISEPSIS was designed, available observational data showed a relative effect size on sepsis mortality in ICU patients taking long-term aspirin prior to ICU admission, in the order of 40 to 80%.[22 25] These data are from two studies with fundamentally different design. One was an observational study of Australian ICU patients, which showed that patients taking aspirin prior to hospitalisation with SIRS who were continued on aspirin had an odds ratio of 0.6 for in-hospital mortality compared with those not treated with aspirin. The hazard ratio calculated from this study for mortality in SIRS patients treated with aspirin was 0.63. The group of patients with proven sepsis had an odds ratio of 0.52 for mortality.[22] The other single centre, German study of aspirin in ICU patients showed reduced mortality (OR 0.19) in patients with an unrestricted range of admission diagnoses.[25]

Using a hazard ratio of 0.63 to examine the sample size required to power our ANTISEPSIS study for the outcome of sepsis-related mortality is a conservative approach given the lower odds ratios available from the German study and Australian patients with proven sepsis. However, we reasoned that the SIRS odds ratio was the most reliable representation of the likely magnitude of aspirin effects on the inflammatory pathways common to SIRS and sepsis given that this figure is based on a large cohort (n=5525) of first SIRS episodes.

The ASPREE study rate of death was estimated to be 17.6 per 1000 participant years. Approximately 4.75 years average follow-up time per participant will be available in an analysis of time to death. We assumed that 20% of the deaths in ASPREE participants would be contributed to by sepsis (as is supported by Australian data [30]). This provides an event

rate for the primary endpoint of ANTISEPSIS of 3.5 per 1000 participant years. Anticipating half of 16,000 Australian ASPREE participants to be randomised to placebo and an event rate of 3.5 per 1000 participant year, we expect 133 primary endpoint events in the placebo group. With this number of sepsis-related deaths, we would have 80% power to detect a hazard ratio of 0.69 for aspirin versus placebo groups. If only 15% of ASPREE deaths are contributed to by sepsis, we would instead expect 100 primary end point events in the control group and still have 80% power to detect a hazard ratio of 0.65. In either of these scenarios, the ANTISEPSIS study is powered to detect an effect less than the hazard ratio of 0.63 measured previously.[26] The two secondary endpoints, admission to hospital for sepsis and admission to ICU for sepsis are more common than death due to sepsis,[3, 4] so they will be powered to detect smaller effects than outlined above for the primary endpoint.

DATA ANALYSIS

We will analyse our primary and two secondary end-points without Bonferroni correction using univariate survival analysis methods; the log-rank test and Cox proportional hazards regression. The proportional hazards assumption will be tested as part of the analysis. In subsequent analysis we will adjust for the following variables known to influence mortality due to sepsis; diabetes, age at recruitment, malignancy, alcohol intake and smoking or chronic lung disease.

The main analyses will compare participant groups as randomised, i.e. be intention to treat. A per protocol analysis will also be performed.

ANTISEPSIS trial results will be reported according to the CONSORT statement.[44]

ETHICAL CONDUCT OF THE TRIAL

This study will be conducted in accordance with the ICH GCP Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) annotated with TGA comments and NH&MRC National Statement on Ethical Conduct in Human Research 2007 protocols, and in keeping with local regulations.

Monash University Human Research Ethics Committee approved the ANTISEPSIS study (CF13/466 – 2013000204, 9 April 2013).

INFORMED CONSENT

The need for individual informed consent for inclusion of ASPREE participants in ANTISEPSIS has been waived by the Monash University Human Research Ethics Committee in its approval of the ANTISEPSIS study. The ANTISEPSIS study conforms to the conditions set out in CHAPTER 2.3: QUALIFYING OR WAIVING CONDITIONS FOR CONSENT in the National Statement on Ethical Conduct in Human Research 2007.

TRIAL REGISTRATION

ANTISEPSIS is registered with the Australian New Zealand Clinical Trials Registry ACTRN12613000349741. The ASPREE study is registered with the International Standard Randomised Controlled Trials Register, ASPirin in Reducing Events in the Elderly, ISRCTN83772183 and clinicaltrials.gov NCT01038583.

STUDY FUNDING

The ANTISEPSIS Study is funded by The National Health & Medical Research Council project grant #1041986 (2013-2017).

DISCUSSION

Primary prevention with aspirin appears to reduce all-cause mortality. A meta analysis of 100,000 aspirin primary prevention trial patient outcomes showed that this reduced all cause mortality (OR, 0.94; 95% CI, 0.88-1.00) and that this reduction was due neither to reduced cardiovascular or cancer deaths.[21] However, deaths due to cancer do appear to be reduced in a meta-analysis of long term follow up of 25,570 participants from primary and secondary prevention aspirin trials. Here the reduction of cancer deaths with aspirin therapy increased with duration of treatment.[45] This finding can therefore be viewed as being separate from the non-cancer reduction of all cause mortality from primary prevention studies. No analysis of non-cardiovascular or non-cancer mortality has been undertaken on data from primary or secondary aspirin prevention studies. It may be that one of the previously unmeasured pathways by which aspirin reduces all-cause mortality is via a reduction in sepsis deaths when aspirin is used for primary prevention. We will test this hypothesis in the ANTISEPSIS trial.

Recruitment to ASPREE was completed in December 2014 with a final number of 16,703 participants in Australia. The collection of information on endpoints will conclude at the end of 2017 and the unblinding of the principal ASPREE trial, and therefore ANTISEPSIS, is expected in mid-2018. If aspirin is shown to be effective in reducing the impact of severe sepsis, it will be the first time that such an outcome has been demonstrated.

ACNOWLEDGEMENTS

The investigators acknowledge the National Institute on Aging (NIA; #RO1-AG029824) for funding the principal ASPREE trial in Australia and the USA, and the National Health & Medical Research Council (grant #334047), the Victorian Cancer Agency and Monash University for funding support of ASPREE in Australia. The investigators also acknowledge the work of all ASPREE field research staff, and those within the ASPREE data team who source the supporting documents from hospitals and pathology services. The investigators would particularly like to acknowledge the valued contribution of the ASPREE participants and the support from their general practitioners.

AUTHORS' CONTRIBUTIONS.

DPE conceived the study.

DPE, KL, ESMcB, DP, RW and RLW wrote the study protocol.

JL designed the database used in the study.

JJM is responsible for the ASPREE study on which this study protocol is based.

ESMcB and RLW designed the statistical analysis plan fort he protocol.

DPE, KL, ESMcB, DP, RW and EM designed the implementation aspects of this protocol.

All authors critically reviewed the manuscript.

COMPETING INTERESTS STATEMENT.

None of the authors have competing interests.

REFERENCES

1. World Health Organisation. The top 10 causes of death. 2014. http://www.who.int/mediacentre/factsheets/fs310/en/index.html.

2. Global Burden of Diseases Study, Mortality, Causes of Death Committee. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 2015;**385**(9963):117-71 doi: 10.1016/S0140-6736(14)61682-2.

3. Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA 2016;**315**(8):801-10 doi: 10.1001/jama.2016.0287.

4. Kaukonen KM, Bailey M, Suzuki S, Pilcher D, Bellomo R. Mortality related to severe sepsis and septic shock among critically ill patients in Australia and New Zealand, 2000-2012. JAMA 2014;**311**(13):1308-16 doi: 10.1001/jama.2014.2637.

5. Cohen J, Vincent JL, Adhikari NK, et al. Sepsis: a roadmap for future research. Lancet Infect Dis 2015;**15**(5):581-614 doi: 10.1016/S1473-3099(15)70112-X.

6. Goodman SG, Menon V, Cannon CP, Steg G, Ohman EM, Harrington RA. Acute ST-segment elevation myocardial infarction: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). Chest 2008;**133**(6 Suppl):708S-75S

7. Eisen DP. Manifold beneficial effects of acetyl salicylic acid and nonsteroidal anti-inflammatory drugs on sepsis. Intensive Care Med 2012;**38**(8):1249-57 doi: 10.1007/s00134-012-2570-8.

8. Kopp E, Ghosh S. Inhibition of NF-kappa B by sodium salicylate and aspirin. Science 1994;**265**(5174):956-9

9. Takada Y, Bhardwaj A, Potdar P, Aggarwal BB. Nonsteroidal anti-inflammatory agents differ in their ability to suppress NF-kappaB activation, inhibition of expression of cyclooxygenase-2 and cyclin D1, and abrogation of tumor cell proliferation. Oncogene 2004;**23**(57):9247-58

10. Serhan CN, Haeggstrom JZ, Leslie CC. Lipid mediator networks in cell signaling: update and impact of cytokines. FASEB J 1996;**10**(10):1147-58

11. Serhan CN, Chiang N. Lipid-derived mediators in endogenous anti-inflammation and resolution: lipoxins and aspirin-triggered 15-epi-lipoxins. Scientific World Journal 2002;**2**:169-204 doi: 10.1100/tsw.2002.81.

12. Arita M, Yoshida M, Hong S, et al. Resolvin E1, an endogenous lipid mediator derived from omega-3 eicosapentaenoic acid, protects against 2,4,6-trinitrobenzene sulfonic acid-induced colitis. Proc Natl Acad Sci USA 2005;**102**(21):7671-6 doi: 10.1073/pnas.0409271102.

13. Paul-Clark MJ, Van Cao T, Moradi-Bidhendi N, Cooper D, Gilroy DW. 15-epi-lipoxin A4-mediated induction of nitric oxide explains how aspirin inhibits acute inflammation. J Exp Med 2004;**200**(1):69-78 doi: 10.1084/jem.20040566.

14. Chiang N, Bermudez EA, Ridker PM, Hurwitz S, Serhan CN. Aspirin triggers antiinflammatory 15-epi-lipoxin A4 and inhibits thromboxane in a randomized human trial. Proc Natl Acad Sci USA 2004;**101**(42):15178-83 doi: 10.1073/pnas.0405445101.

15. El Kebir D, Jozsef L, Pan W, et al. 15-epi-lipoxin A4 inhibits myeloperoxidase signaling and enhances resolution of acute lung injury. Am J Respir Crit Care Med 2009;**180**(4):311-9 doi: 10.1164/rccm.200810-1601OC.

16. Hennekens CH, Schneider WR, Pokov A, et al. A randomized trial of aspirin at clinically relevant doses and nitric oxide formation in humans. J Cardiovasc Pharmacol Ther 2010;**15**(4):344-8 doi: 10.1177/1074248410375091.

17. Morris T, Stables M, Hobbs A, et al. Effects of low-dose aspirin on acute inflammatory responses in humans. J Immunol 2009;**183**(3):2089-96 doi: jimmunol.0900477 [pii] 10.4049/jimmunol.0900477.

18. Cox D, Kerrigan SW, Watson SP. Platelets and the innate immune system: mechanisms of bacterial-induced platelet activation. J Thromb Haemost 2011;**9**(6):1097-107 doi: 10.1111/j.1538-7836.2011.04264.x.

19. Masotti G, Galanti G, Poggesi L, Abbate R, Neri Serneri GG. Differential inhibition of prostacyclin production and platelet aggregation by aspirin in humans. Adv Prostaglandin Thromboxane Res 1980;**6**:317-20

20. Huang ES, Strate LL, Ho WW, Lee SS, Chan AT. Long-term use of aspirin and the risk of gastrointestinal bleeding. Am J Med 2011;**124**(5):426-33 doi: 10.1016/j.amjmed.2010.12.022.

21. Seshasai SR, Wijesuriya S, Sivakumaran R, et al. Effect of aspirin on vascular and nonvascular outcomes: meta-analysis of randomized controlled trials. Arch Intern Med 2012;**172**(3):209-16 doi: 10.1001/archinternmed.2011.628.

22. Eisen DP, Reid D, McBryde ES. Acetyl salicylic acid usage and mortality in critically ill patients with the systemic inflammatory response syndrome and sepsis. Crit Care Med 2012;**40**(6):1761-7 doi: 10.1097/CCM.0b013e318246b9df.

23. Lösche W BJ, Kabisch B, Winning J, Claus RA, Bauer M. . Do aspirin and other antiplatelet drugs reduce the mortality in critically ill patients? Thrombosis 2011(2012)

24. Winning J, Neumann J, Kohl M, et al. Antiplatelet drugs and outcome in mixed admissions to an intensive care unit. Crit Care Med 2010;**38**(1):32-7 doi: 10.1097/CCM.0b013e3181b4275c.

25. Winning J, Reichel J, Eisenhut Y, et al. Anti-platelet drugs and outcome in severe infection: clinical impact and underlying mechanisms. Platelets 2009;**20**(1):50-7 doi: 908188675 [pii] 10.1080/09537100802503368.

26. Chen W JD, Bastarache JA, May AK, O'Neal Jr HR, Bernard GR, et al. Prehospital aspirin use is associated with reduced risk of acute respiratory distress syndrome in critically ill patients: a propensity-adjusted analysis. Crit Care Med 2015;**43**(4):801-07

27. Otto GP SM, Boettel J, Kabisch B, Breuel H, Winning J, et al. Effects of low-dose acetylsalicylic acid and atherosclerotic vascular diseases on the outcome in patients with severe sepsis or septic shock. Platelets 2013;**24**(6):480-85

28. Valerio-Rojas JC, Jaffer IJ, Kor DJ, Gajic O, Cartin-Ceba R. Outcomes of severe sepsis and septic shock patients on chronic antiplatelet treatment: a historical cohort study. Crit Care Res Pract 2013;**2013**:782573 doi: 10.1155/2013/782573.

29. Boyle AJ, Di Gangi S, Hamid UI, et al. Aspirin therapy in patients with acute respiratory distress syndrome (ARDS) is associated with reduced intensive care unit mortality: a prospective analysis. Crit Care 2015;**19**:109 doi: 10.1186/s13054-015-0846-4.

30. Falcone M RA, Cangemi R, Farcomeni A, Calvieri C, Barillà F, et al. . Lower mortality rate in elderly patients with community-onset pneumonia on treatment with aspirin. J Am Heart Assoc 2015;**4**(1):e001595

31. Sossdorf M OG, Boettel J, Winning J, Lösche W. Benefit of low-dose aspirin and non-steroidal anti-inflammatory drugs in septic patients. Crit Care 2013;**17**(1):1-2

32. Tsai MJ, Ou SM, Shih CJ, et al. Association of prior antiplatelet agents with mortality in sepsis patients: a nationwide population-based cohort study. Intensive Care Med 2015;**41**(5):806-13 doi: 10.1007/s00134-015-3760-y.

33. Al Harbi SA, Tamim HM, Al-Dorzi HM, Sadat M, Arabi YM. Association between aspirin therapy and the outcome in critically ill patients: a nested cohort study. BMC Pharmacol Toxicol 2016;**17**:5 doi: 10.1186/s40360-016-0047-z.

34. Wiewel MA, de Stoppelaar SF, van Vught LA, et al. Chronic antiplatelet therapy is not associated with alterations in the presentation, outcome, or host response biomarkers during sepsis: a propensity-matched analysis. Intensive Care Med 2016;**42**(3):352-60 doi: 10.1007/s00134-015-4171-9.

35. ASPREE Investigators Group. Study design of ASPirin in Reducing Events in the Elderly (ASPREE): a randomized, controlled trial. Contemp Clin Trials 2013;**36**(2):555-64 doi: 10.1016/j.cct.2013.09.014.

36. Bone RC, Balk RA, Cerra FB, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. Chest 1992;**101**(6):1644-55

37. Calandra T, Cohen J, International Sepsis Forum Definition of Infection in the ICUCC. The international sepsis forum consensus conference on definitions of infection in the intensive care unit. Crit Care Med 2005;**33**(7):1538-48

38. Mandell LA, Wunderink RG, Anzueto A, et al. Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. Clin Infect Dis 2007;**44 Suppl 2**:S27-72 doi: 10.1086/511159.

39. Tablan OC, Anderson LJ, Besser R, et al. Guidelines for preventing health-care--associated pneumonia, 2003: recommendations of CDC and the Healthcare Infection Control Practices Advisory Committee. MMWR Recomm Rep 2004;**53**(RR-3):1-36

40. Centers for Disease Control. CDC/NHSN Surveillance Definitions for Specific Types of Infections. Secondary CDC/NHSN Surveillance Definitions for Specific Types of Infections 2016. http://www.cdc.gov/nhsn/PDFs/pscManual/17pscNosInfDef_current.pdf.

41. Centers for Disease Control. Urinary Tract Infection (Catheter-Associated Urinary Tract Infection [CAUTI] and Non-Catheter-Associated Urinary Tract Infection [UTI]) and Other Urinary System Infection [USI]) Events. Secondary Urinary Tract Infection (Catheter-Associated Urinary Tract Infection [CAUTI] and Non-Catheter-Associated Urinary Tract Infection [UTI]) and Other Urinary System Infection [USI]) Events 2016. http://www.cdc.gov/nhsn/PDFs/pscManual/17pscNosInfDef_current.pdf.

42. Gunderson CG. Cellulitis: definition, etiology, and clinical features. Am J Med 2011;**124**(12):1113-22 doi: 10.1016/j.amjmed.2011.06.028.

43. Li JS, Sexton DJ, Mick N, et al. Proposed modifications to the Duke criteria for the diagnosis of infective endocarditis. Clin Infect Dis 2000;**30**(4):633-8 doi: 10.1086/313753.

44. Moher D, Hopewell S, Schulz KF, et al. CONSORT 2010 Explanation and Elaboration: Updated guidelines for reporting parallel group randomised trials. J Clin Epidemiol 2010;**63**(8):e1-37 doi: 10.1016/j.jclinepi.2010.03.004.

45. Rothwell PM, Fowkes FG, Belch JF, Ogawa H, Warlow CP, Meade TW. Effect of daily aspirin on long-term risk of death due to cancer: analysis of individual patient data

from randomised trials. Lancet 2011;**377**(9759):31-41 doi: 10.1016/S0140-6736(10)62110-1.

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	7-8 (abstract) 22
	2b	All items from the World Health Organization Trial Registration Data Set	3-6
Protocol version	3	Date and version identifier	2
Funding	4	Sources and types of financial, material, and other support	22
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1-2
	5b	Name and contact information for the trial sponsor	Non commercial trial
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	Non commercial trial
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	19

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	Hypothesis 13 Justification, background, risks 10-12
	6b	Explanation for choice of comparators	13
Objectives	7	Specific objectives or hypotheses	13
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	13
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	14(reference to parent study)
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	14(reference to parent study)
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	14(reference to parent study)
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	14(reference to parent study)
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	14(reference to parent study)
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	14(reference to parent study)

Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	16-19
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	14, 23
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	20-21
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	14 (reference to parent study)
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	14
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	14(reference to parent study)
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	14(reference to parent study)
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	13, 19
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	14(reference to parent study)
Methods: Data collection, management, and analysis			

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	14(reference to parent study)
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	14(reference to parent study)
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	14(reference to parent study)
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	21
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	21
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	21
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	19
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	14(reference to parent study)
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	14(reference to parent study)
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	14(reference to parent study)
Ethics and dissemination			

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	22
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	14(reference to parent study)
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	22
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	Not applicable
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	14(reference to parent study)
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	24
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	Not applicable
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	14(reference to parent study)
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	14(reference to parent study)
	31b	Authorship eligibility guidelines and any intended use of professional writers	14(reference to parent study)
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	14(reference to parent study)

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Consent waived, not applicable
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	Not applicable

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated.