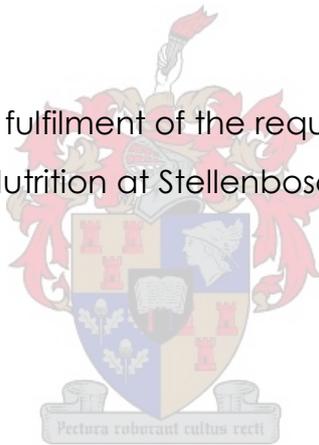


**MICRONUTRIENT SUPPLEMENTATION  
FOR CRITICALLY ILL ADULTS:  
A SYSTEMATIC REVIEW OF THE EVIDENCE**

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Thesis presented in partial fulfilment of the requirements for the degree of  
Master of Nutrition at Stellenbosch University



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**Study Co-leader** : Dr R Blaauw

**December 2008**

## DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the owner of the copyright thereof and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Signature:

A handwritten signature in black ink that reads "J. Visser". The signature is written in a cursive style with a long horizontal line extending from the bottom of the "s".

Date: December 2008

# ABSTRACT

## **Background**

Critical illness is associated with increased production of reactive oxygen species and oxidative stress, and low levels of most micronutrients with resultant diminished endogenous antioxidant defences. Micronutrient supplementation is thought to be beneficial to the critically ill patient by ameliorating oxidative stress and by improving clinical outcome.

## **Objectives**

This systematic review assessed the effects of micronutrient supplementation on adults recovering from critical illness. Primary outcomes included clinical endpoints [mortality, infectious complications, length of intensive care unit and hospital stay (LICU and LOS)]. Secondary outcomes included descriptions of practice issues, micronutrient status, morbidity, course of the acute phase response and oxidative stress.

## **Search strategy**

An electronic bibliographic database search was carried out, bibliographies of retrieved articles were reviewed and personal files searched to obtain additional citations. Databases were searched from inception until 29 February 2008.

## **Selection criteria**

Randomized controlled trials (RCTs) of micronutrient supplementation (by any route) in adult critically ill patients, given in addition to their routine care, were included.

## **Data collection and analysis**

Two authors independently extracted data and assessed trial quality. For the primary outcomes the random-effects model was used to estimate overall relative risk / mean difference and effect size due to the presence of study heterogeneity. Selected exploratory analyses were undertaken. Differences at the level of  $p < 0.05$  was considered to be statistically significant. The secondary outcomes were sparse and variably recorded such that this data was not formally aggregated.

## **Main results**

Fifteen RCTs involving 1714 participants and 18 RCTs involving 1849 participants were included for the primary and secondary objectives respectively. The quality of the RCTs, as reported, was disappointing, particularly for allocation concealment. Fourteen trials ( $n=1468$ ) of micronutrient supplementation showed a statistically

significant reduction in overall mortality [relative risk (RR) 0.78, 95% confidence interval (CI) 0.67-0.90,  $I^2=0\%$ ,  $p=0.0009$ ]. An asymmetrical funnel plot necessitates caution when directly interpreting these results. Six RCTs ( $n=1194$ ) indicated a statistically significant reduction in 28 day mortality (RR 0.75, 95% CI 0.63-0.88,  $I^2=0\%$ ,  $p=0.0006$ ) (symmetrical funnel plot). Micronutrient supplementation in this systematic review was not associated with a reduction in infectious complications, LICU or LOS. In sub-group analyses, single nutrients were associated with borderline statistical significance (RR 0.82, 95% CI 0.66-1.01,  $I^2=0\%$ ,  $p=0.06$ ) in terms of mortality, whilst a sensitivity analysis of combined micronutrients indicated a significant reduction in mortality (RR 0.69, 95% CI 0.54-0.90,  $I^2=2\%$ ,  $p=0.006$ ). This review did not find clear evidence that parenteral is superior to enteral administration in terms of clinical outcomes. The secondary outcomes confirmed that timing, duration and dosing are key factors to ensure optimal clinical benefit.

### **Conclusion**

This review does suggest potential benefit of micronutrient supplementation in critically ill adults for some clinical outcomes (especially mortality), but also highlights that caution is warranted as nutrient interactions and risk of toxicity are not clearly defined in critical illness. More large multi-centre randomized trials are necessary to assess the effects of different types and doses of micronutrient supplementation in selected groups of patients with different types of critical illness.

# OPSOMMING

## **Agtergrond**

Kritieke siekte word geassosieer met verhoogde produksie van reaktiewe suurstof spesies en oksidatiewe stres, sowel as lae vlakke van mikronutriënte, met gevolglike ingekorte endogene antioksidant verdedigingsmeganismes. Mikronutriënt suplementasie is moontlik voordelig vir kritieke siek pasiënte deur die verbetering van oksidatiewe stres en kliniese uitkoms.

## **Doelwitte**

Hierdie sistematiese literatuuroorsig het die effek van mikronutriënt suplementasie op volwassenes, wat van kritieke siekte herstel, geassesseer. Primêre uitkomst is ingesluit kliniese eindpunte (mortaliteit, infektiewe komplikasies, lengte van intensiewesorg-eenheid en hospitaal verblyf). Sekondêre uitkomst is ingesluit beskrywings van praktyk-verwante aspekte, mikronutriënt status, morbiditeit, verloop van die akute fase respons en oksidatiewe stres.

## **Soek strategie**

'n Elektroniese bibliografiese databasis soektog was uitgevoer, bibliografieë van artikels was hersien en persoonlike lêers is nagegaan om addisionele verwysings te verkry. Soektogte het gestrek van databasis-aanvangs tot 29 Februarie 2008.

## **Seleksie kriteria**

Verwekansigde gekontroleerde proewe (RCTs) van mikronutriënt suplementasie (via enige roete) in volwasse kritieke siek pasiënte, wat gegee word addisioneel tot roetine sorg, was ingesluit.

## **Data versameling en analise**

Twee outeurs het onafhanklik data ekstraksie onderneem en proef kwaliteit geassesseer. Vir die primêre uitkomst is die stogastiese-effekte model gebruik om algehele relatiewe risiko / gemiddelde verskil en effek groter te skat as gevolg van die teenwoordigheid van studie heterogeniteit. Geselekteerde ondersoekende analises was onderneem. Verskille was statisties beduidend geag indien  $p < 0.05$  was. Die sekondêre uitkomst was skaars en veranderlik gerapporteer tot so 'n mate dat data nie formeel saamgevoeg is nie.

## **Hoof resultate**

Fyftien RCTs met 1714 pasiënte en 18 RCTs met 1849 pasiënte was ingesluit vir die primêre en sekondêre uitkomst onderskeidelik. Die kwaliteit van die RCTs was

teleurstellend, veral vir toedeling verberging. Veertien proewe (n=1468) het 'n statisties beduidende verlaging in algehele mortaliteit aangetoon [relatiewe risiko (RR) 0.78, 95% vertrouensinterval (VI) 0.67-0.90,  $I^2=0\%$ ,  $p=0.0009$ ]. 'n Asimetriese tregterstipping dui aan dat versigtige interpretasie van hierdie resultaat noodsaaklik is. Ses RCTs (n=1194) het 'n statisties beduidende verlaging in 28-dag mortaliteit aangetoon (RR 0.75, 95% VI 0.63-0.88,  $I^2=0\%$ ,  $p=0.0006$ ) (simmetriese tregterstipping). Mikronutriënt suplementasie in hierdie sistematiese oorsig was nie geassosieer met 'n verlaging in infektiewe komplikasies, lengte van intensiewesorg-eenheid en hospitaal verblyf nie. In sub-groep analyses was enkel nutriënte geassosieer met grenslyn statistiese beduidenheid in terme van mortaliteit (RR 0.82, 95% VI 0.66-1.01,  $I^2=0\%$ ,  $p=0.06$ ), terwyl 'n sensitiwiteitsanalise van gekombineerde mikronutriënte 'n beduidende verlaging in mortaliteit aangetoon het (RR 0.69, 95% VI 0.54-0.90,  $I^2=2\%$ ,  $p=0.006$ ). Hierdie oorsig het nie duidelike bewyse gevind dat parenteraal superieur is tot enterale administrasie in terme van kliniese uitkomst nie. Die sekondêre uitkomst het bevestig dat tydsberekening, duurte en dosering sleutel faktore is om optimale kliniese voordeel te verseker.

### **Gevolgtrekking**

Hierdie oorsig dui op die potensiële voordeel van mikronutriënt suplementasie in kritieke siek volwassenes vir sommige kliniese uitkomst (veral mortaliteit), maar beklemtoon dat versigtigheid noodsaaklik is aangesien nutriënt interaksies en risiko vir toksisiteit nie duidelik gedefinieer is in kritieke siekte nie. Meer groot, multi-sentra proewe is nodig om die effek van verskillende tipes en dosisse van mikronutriënt suplementasie in geselekteerde groepe van pasiënte met verskillende tipes kritieke siekte te assesser.

## ACKNOWLEDGEMENTS

I am indebted to the following people who in various ways (directly and indirectly) contributed to the completion of this project.

### ***Thanks and appreciation to:***

- Prof Demetre Labadarios, an exceptional study leader and role model whose vast knowledge and commitment to science, education and nutrition is admirable and a great inspiration. His support and guidance continue to form an integral part of my professional development.
- Dr Reneé Blaauw, an outstanding co-study leader and inspiration to many a dietitian, including me, for her recognized expertise and professional conduct.
- My two excellent reviewers, dietitians Louise Lombard and Carla Labuschagne for their very efficient and accurate work.
- Statistician Martie Muller, for her quick and professional assistance with the statistical analyses.
- Librarians Ingrid van der Westhuizen and Elsie Geldenhuys for their friendly and efficient assistance.
- Prof Jimmy Volmink, for his expert advice in a time of need.
- Prof Marietjie Herselman, Head: Division Human Nutrition for her continued encouragement, support and belief in the development of “her people”.
- My colleagues and friends, Debbi Marais and Maritha Marais whose work ethic, loyalty and support I admire and greatly appreciate.

### ***And on a personal note, my heartfelt thank you to:***

- My husband Jacqué, who wholeheartedly supports and encourages my goals and educational endeavours.
- My son Stefan, in whom I hope to instill the value of learning and who I hope will strive for excellence in all he pursues.
- My parents, Careen and Pikkie Conradie, who taught me to appreciate the opportunity to learn and who provided the platform to realize my potential.
- My sister, Nelene Conradie, for being a best friend, confidant and rock to lean on.
- My parents' in-law, Lynette and Kobus Visser for their generous assistance and support that contributed to the completion of this project.

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## LIST OF ABBREVIATIONS

<b>AMA</b>	American medical association	<b>NF-<math>\kappa</math>B</b>	Nuclear factor-kappa B
<b>AOX</b>	Antioxidant	<b>NO</b>	Nitric oxide
<b>APACHE</b>	Acute physiology and chronic health evaluation	<b>NS</b>	Not significant
<b>APR</b>	Acute phase response	<b>PLP</b>	Pyridoxal-5'-phosphate
<b>ARDS</b>	Adult respiratory distress syndrome	<b>PN</b>	Parenteral nutrition
<b>CARS</b>	Compensatory anti-inflammatory response syndrome	<b>PO</b>	Per os
<b>CI</b>	Confidence interval	<b>RBC</b>	Red blood cell
<b>CRP</b>	C-reactive protein	<b>RBP</b>	Retinol binding protein
<b>Cu</b>	Copper	<b>RCT</b>	Randomized controlled trial
<b>EN</b>	Enteral nutrition	<b>RDA</b>	Recommended dietary allowance
<b>Fe</b>	Iron	<b>REE</b>	Resting energy expenditure
<b>FFA</b>	Free fatty acid	<b>RevMan</b>	Review manager
<b>GCS</b>	Glasgow Coma Scale	<b>ROI</b>	Reactive oxygen intermediate
<b>GSH</b>	Glutathione	<b>ROS</b>	Reactive oxygen species
<b>GSHPx</b>	Selenoenzyme glutathione peroxidase	<b>RR</b>	Risk ratio / relative risk
<b>GSSG</b>	Glutathione disulphide	<b>Rx</b>	Treatment / experimental
<b>ICU</b>	Intensive care unit	<b>SAPS</b>	Simplified acute physiology score
<b>IFN</b>	Interferon	<b>SD</b>	Standard deviation
<b>IL</b>	Interleukin	<b>Se</b>	Selenium
<b>ISS</b>	Injury severity score	<b>SE</b>	Standard error
<b>ITT</b>	Intention-to-treat	<b>SOFA</b>	Sequential organ failure assessment
<b>IV</b>	Intravenous	<b>SIRS</b>	Systemic inflammatory response syndrome
<b>LICU</b>	Length of intensive care unit stay	<b>SOD</b>	Superoxide dismutase
<b>LODS</b>	Logistic organ dysfunction score	<b>TAC</b>	Total antioxidant capacity
<b>LOS</b>	Length of hospital stay	<b>TBARS</b>	Thiobarbituric acid reactive substances
<b>MD</b>	Mean difference	<b>TNF</b>	Tumor necrosis factor
<b>MDA</b>	Malondialdehyde	<b>VLDL</b>	Very low density lipoprotein
<b>MN</b>	Micronutrient	<b>WBC</b>	White blood cell
<b>MODS</b>	Multiple organ dysfunction score	<b>Zn</b>	Zinc

## LIST OF DEFINITIONS

**Blinding:**<sup>1</sup> The process of preventing those involved in a trial from knowing to which comparison group a particular participant belongs.

**Concealment of allocation:**<sup>1</sup> The process used to ensure that the person deciding to enter a participant into a randomized controlled trial does not know the comparison group into which that individual will be allocated to. This is distinct from blinding, and is aimed at preventing selection bias.

**Confidence interval (CI):**<sup>1</sup> A measure of the uncertainty around the main finding of a statistical analysis.

**Continuous data:**<sup>1</sup> Data with a potentially infinite number of possible values within a given range.

**Critically ill:** For the purpose of this systematic review "critically ill patients" includes medical, surgical and burns patients treated in an Intensive Care Unit (ICU) (as defined by the investigators).

**Dichotomous data:**<sup>1</sup> Data that can take one of two possible values, such as dead/alive, smoker/non-smoker, present/not present (also called binary data).

**Forest plot:**<sup>1</sup> A graphical representation of the individual results of each study included in a meta-analysis together with the combined meta-analysis result. The plot also allows readers to see the heterogeneity among the results of the studies. The overall estimate from the meta-analysis and its confidence interval are shown at the bottom, represented as a diamond. The centre of the diamond represents the pooled point estimate, and its horizontal tips represent the confidence interval.

**Funnel plot:**<sup>1</sup> A graphical display of some measure of study precision plotted against effect size that can be used to investigate whether there is a link between study size and treatment effect. One possible cause of an observed association is reporting bias.

**Heterogeneity:**<sup>1</sup> 1. Used in a general sense to describe the variation in, or diversity of, participants, interventions, and measurement of outcomes across a set of studies, or the variation in internal validity of those studies. 2. Used specifically, as statistical heterogeneity, to describe the degree of variation in the effect estimates from a set of studies. Also used to indicate the presence of variability among studies beyond the amount expected due solely to the play of chance. See also I<sup>2</sup>.

**I<sup>2</sup>:**<sup>1</sup> A measure used to quantify heterogeneity. It describes the percentage of the variability in effect estimates that is due to heterogeneity rather than sampling error (chance). A value greater than 50% may be considered to represent substantial heterogeneity.

**Intention-to-treat analysis:**<sup>1</sup> A strategy for analyzing data from a randomized controlled trial. All participants are included in the arm to which they were allocated, whether or not they received (or completed) the intervention given to that arm. Intention-to-treat analysis prevents bias caused by the loss of participants, which may disrupt the baseline equivalence established by randomization and which may reflect non-adherence to the protocol. The term is often misused in trial publications when some participants were excluded.

**Meta-analysis:**<sup>1</sup> The use of statistical techniques in a systematic review to integrate the results of included studies.

**Methodological quality:**<sup>1</sup> The extent to which the design and conduct of a study are likely to have prevented bias. Variation in quality can explain variation in the results of studies included in a systematic review. More rigorously designed (better quality) trials are more likely to yield results that are closer to the truth (also called internal validity but better thought of as relating to bias prevention).

**Micronutrient supplementation:** For the purpose of this systematic review “micronutrient supplementation” refers to the administration of micronutrients (enterally and/or parenterally) over and above the micronutrients already included in the enteral / parenteral formula(s) the patient is receiving as part of normal nutritional support (as defined by the investigators).

**Narrative review:**<sup>1</sup> A review article in the medical literature which summarizes a number of different studies and may draw conclusions about a particular intervention. Narrative review articles are not systematic. Also referred to as overviews.

**Per-protocol analysis:**<sup>1</sup> An analysis of the subset of participants from a randomized controlled trial who complied with the protocol sufficiently to ensure that their data would be likely to exhibit the effect of treatment. This subset may be defined after considering exposure to treatment, availability of measurements and absence of major protocol violations. The per protocol analysis strategy may be subject to bias as the reasons for noncompliance may be related to treatment.

**Quasi-random allocation:**<sup>1</sup> Methods of allocating people to a trial that are not random, but were intended to produce similar groups when used to allocate participants. Quasi-random methods include: allocation by the person's date of birth, by the day of the week or month of the year, by a person's medical record number, or just allocating every alternate person. In practice, these methods of allocation are relatively easy to manipulate, introducing selection bias.

**Random-effects model:**<sup>1</sup> A statistical model in which both within-study sampling error (variance) and between studies variation are included in the assessment of the uncertainty (confidence interval) of the results of a meta-analysis. When there is heterogeneity among the results of the included studies beyond chance, random-effects models will give wider confidence intervals than fixed-effect models.

**Randomization:**<sup>1</sup> The process of randomly allocating participants into one of the arms of a controlled trial. There are two components to randomization: the generation of a random sequence, and its implementation, ideally in a way so that those entering participants into a study are not aware of the sequence (concealment of allocation).

**Randomized controlled trial:**<sup>1</sup> A study in which two or more interventions, possibly including a control intervention or no intervention, are compared by being randomly allocated to participants.

**RevMan (Review Manager):**<sup>1</sup> Software developed for The Cochrane Collaboration to assist reviewers in preparing Cochrane Reviews and systematic reviews in general.

**Risk ratio (RR):**<sup>1</sup> The ratio of risks in two groups. In intervention studies, it is the ratio of the risk in the intervention group to the risk in the control group. A risk ratio of one indicates no difference between comparison groups. For undesirable outcomes, a risk ratio that is less than one indicates that the intervention was effective in reducing the risk of that outcome. (Also called relative risk.)

**Sensitivity analysis:**<sup>1</sup> An analysis used to determine how sensitive the results of a study or systematic review are to changes in how it was done. Sensitivity analyses are used to assess how robust the results are to uncertain decisions or assumptions about the data and the methods that were used.

**Sub-group analysis:**<sup>1</sup> An analysis in which the intervention effect is evaluated in a defined subset of the participants in a trial, or in complementary subsets, such as by sex or in age categories.

**Systematic review:**<sup>1</sup> A review of a clearly formulated question that uses systematic and explicit methods to identify, select, and critically appraise relevant research, and to collect and analyze data from the studies that are included in the review. Statistical methods (meta-analysis) may or may not be used to analyze and summarize the results of the included studies.

## **CHAPTER 1: LITERATURE REVIEW AND MOTIVATION FOR THE STUDY**

## **1.1 INTRODUCTION**

It is increasingly and more consistently realized that micronutrients have an important role to play in health and disease. Apart from the prevention of clinical deficiency syndromes, the fast developing field of immunonutrition has added yet another dimension to the importance of foods, nutrition and micronutrients in disease prevention and therapy. It is well known that micronutrients are involved in the prevention of nutritional deficiencies, immune humoral and cellular defence, regulation of gene expression during the acute phase response, antioxidant defence and prevention of chronic diseases.<sup>2</sup> Despite these major developments on the importance of other aspects of micronutrient status, understanding of the exact role of the latter in critical illness remains elusive and ill defined, complicating decision-making on the part of the nutrition support practitioner.

Successful clinical decisions, like most human decisions, are complex in nature. In making them, we draw on information from many different sources, including primary data and patients preferences, our own clinical and personal experience, external rules and constraints, and, importantly, scientific evidence.<sup>3</sup> For the latter, the evidence-based approach has been advocated and implemented to interpret the vast body of available scientific literature. The phrase "evidence-based nutrition" has been defined as "the application of the best available systematically assembled evidence in setting nutrition policy and practice".<sup>4</sup> Systematic reviews and meta-analyses are key elements of evidence-based healthcare and are carried out to generate answers to focused questions about health care and related issues.

In the critical care field it is well-known that nutrition support is a key component of therapy in these patients. Although great pains are taken to provide adequate and optimal carbohydrate, lipid and protein combinations, the vital role of micronutrients (vitamins and trace elements) should not be overlooked.<sup>5</sup> Despite the lack of clear guidelines regarding micronutrient requirements in the critically ill, a growing body of evidence (though conflicting at times) is emerging. To make sense of this information an evidence-based approach, i.e. a systematic review of the evidence is warranted and could provide further clarity on the use of micronutrients in critical care.

To not pre-empt the literature associated with micronutrient supplementation in critical care and thus the outcome of this systematic review, the literature overview will focus on the concepts of evidence-based nutrition (specifically the processes associated with conducting systematic reviews) and as background the effect of the inflammatory response on micronutrient status.

## **1.2 EVIDENCE-BASED NUTRITION**

The terms "systematic review" and "meta-analysis" (key components of evidence-based nutrition) are often, incorrectly, used interchangeably. Egger and Smith<sup>6</sup> suggest that the term *meta-analysis* should be used to "describe the statistical integration of separate studies whereas *systematic review* is most appropriate for denoting any review of a body of data that uses clearly defined methods and criteria". This definition suggests that a meta-analysis can, if appropriate, be part of a systematic review. Simply put, a systematic review may have a statistical combination of studies (a meta-analysis), but only if appropriate and possible with the available data.

### **1.2.1 Systematic Reviews and Meta-Analyses**

A review earns the adjective systematic if it is based on a clearly formulated question(s), identifies relevant literature, assess their quality, summarize the evidence by use of explicit methodology and interpret the findings in a logical and practical manner.<sup>7</sup> They synthesize the results of multiple primary investigations by using strategies that limit bias and random error.<sup>8,9</sup>

A clear distinction should be made between systematic reviews and narrative (traditional) reviews. Although all reviews are retrospective, observational research studies and therefore subject to systematic and random error, systematic reviews employ clearly-defined scientific review methods to minimize error and bias.<sup>10,11</sup> This is the key feature that distinguishes traditional narrative reviews from systematic reviews (Table 1.1).

**Table 1.1: Differences between traditional narrative reviews and systematic reviews\***

<b>Feature</b>	<b>Narrative (traditional) review</b>	<b>Systematic review</b>
<b>Question</b>	Broad in scope	Focused, clearly formulated question(s)
<b>Sources &amp; search</b>	Not necessarily specified, potentially biased	Comprehensive sources & clearly defined search strategy
<b>Selection</b>	Not necessarily specified, potentially biased	Strictly criterion-based selection
<b>Appraisal</b>	Variable	Strict / meticulous critical appraisal
<b>Synthesis</b>	Often qualitative summary	Quantitative summary (if appropriate)
<b>Conclusion(s)</b>	Sometimes evidence-based	Evidence-based

\* Source: Adapted from reference<sup>10</sup>

Due to large volumes of medical literature and time constraints, practitioners have reported that they often prefer summaries of information to publications of original investigations.<sup>12</sup> It has been stated that the results of single studies (although important) are of more limited value in terms of evidence-based practice; instead, syntheses of results of studies are the appropriate product of research.<sup>13</sup> Well-conducted systematic reviews are invaluable to practitioners, investigators and administrators as it provides a scientifically sound summary of the available literature. Systematic reviews can be used at bedside (practitioners),<sup>4</sup> to summarize existing data, refine hypotheses, estimate sample sizes and help define future research agendas (investigators),<sup>10</sup> and to develop clinical guidelines, assist in evidence-based policy making and for economic evaluation (administrators, health professionals).<sup>4,10</sup> Despite the obvious benefits of systematic reviews, it should be noted that they can aid, but never replace judgement and sound clinical reasoning (the latter being based on a combination of analogy, experience, heuristics, and theory *as well as* research evidence).<sup>10,14-16</sup>

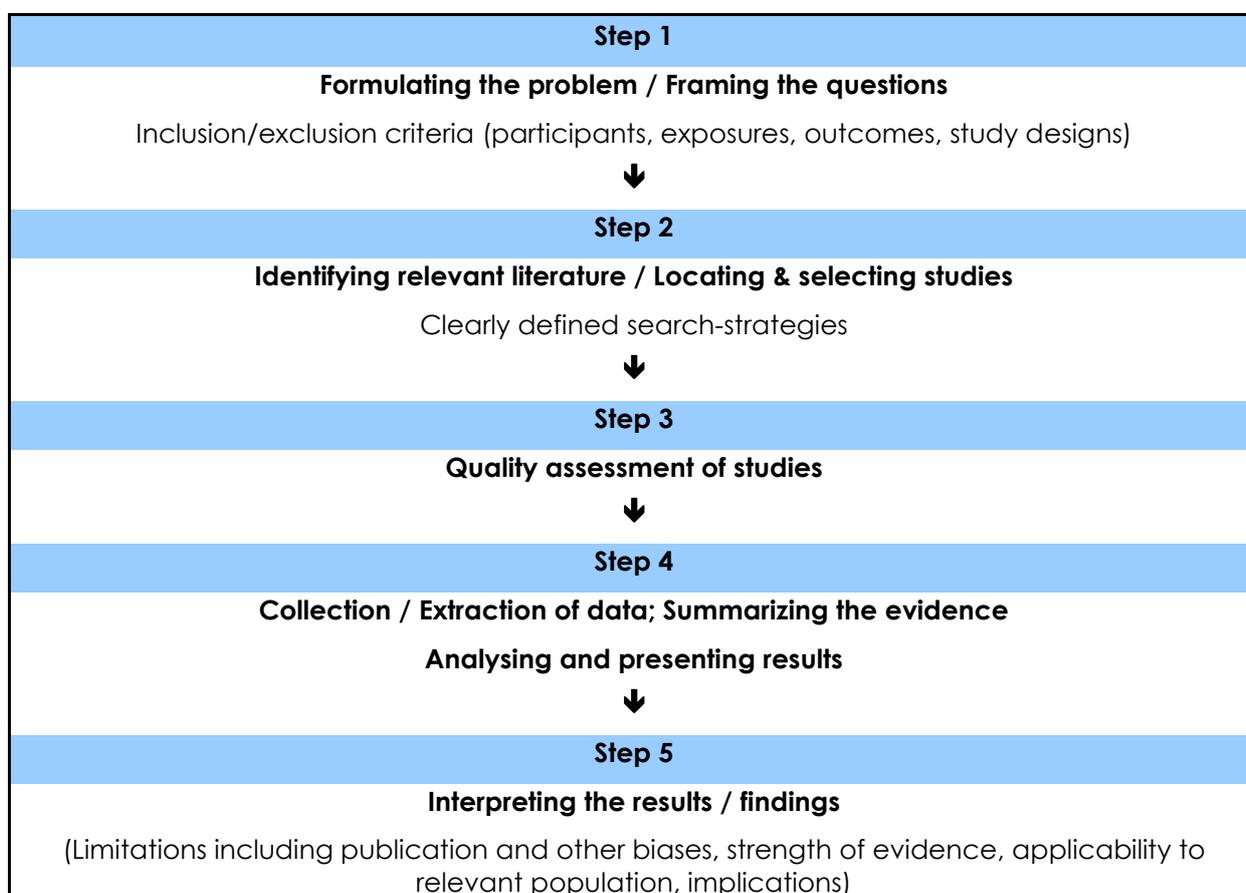
The stages associated with planning the review are:

1. Identification of the need for a systematic review
2. Development of a review protocol

A review protocol specifies the methods that will be used to undertake a specific systematic review. A pre-defined protocol is necessary to reduce the possibility of researcher bias. The components of a protocol include all the elements of the review plus additional planning information.<sup>17</sup>

The steps to conducting a systematic review have been described by various authors, and are summarized in Table 1.2.<sup>4,7,11</sup>

**Table 1.2: The steps in conducting a systematic review\***



\* Source: Adapted from references<sup>7,11</sup>

### **1.2.1.1 Step 1: Formulating the problem / Framing the questions**

The problems to be addressed by the systematic review should be specified in the form of clear, unambiguous and structured questions before beginning the review work.<sup>7,11</sup> Most serious reviewers devote a substantial amount of time and effort in getting the questions right before embarking on the review. A structured approach to framing questions, which uses four components or facets are often used and these

components include the *populations, interventions (or exposures), outcomes* related to the problem statement, and the *study designs* that are suitable for addressing it.<sup>11,18</sup>

### **1.2.1.2 Step 2: Identifying relevant literature / Locating and selecting studies**

The search for studies should be extensive and a clearly defined search strategy (specifying data sources and keywords for searching) should be developed and documented.<sup>7</sup> Multiple resources (including electronic databases, hand-searching, checking reference lists, checking other reviews, print versions of electronic databases, identifying unpublished studies and evidence on adverse effects) should ideally be searched, bearing in mind budget and manpower constraints.<sup>11</sup> It is generally for reviewers to decide which study design(s) to include in their review, but most systematic reviews (and specifically Cochrane reviews) include only randomized or quasi-randomized trials, while others are less restrictive, particularly when few randomized trials addressing the topic of the review are identified.<sup>11</sup> The type of study design to include also rely heavily on the questions posed, and subsequently the types of study designs employed to answer certain research questions. The first stage of checking the results of a search involves assessing titles and abstracts to determine whether each article might meet predetermined eligibility criteria.<sup>11</sup> The latter should flow directly from the review questions, and reasons for inclusion/exclusion of studies should be recorded.<sup>7</sup> Reviewers must decide if more than one of them will assess the records retrieved by electronic databases. There is evidence that using at least two reviewers has an important effect on reducing the possibility that relevant reports will be discarded.<sup>19</sup> Once the screening process has been completed, the full text of the citations considered relevant for the review are retrieved.<sup>11</sup> Upon retrieval of the relevant articles, the predetermined inclusion and exclusion criteria are again applied to the full reference of all the studies to aid final selection. A blinded review process (i.e. reviewers independently reviewing the articles) is recommended by some authors to ensure that no relevant studies are excluded. It is recommended that disagreements regarding inclusion/exclusion of articles be resolved by discussion or a third reviewer.<sup>11</sup>

Biases in publication, location (including English language bias, database bias, citation bias, multiple publication bias and bias in provision of data), and inclusion

are potentially serious problems in systematic reviews and meta-analyses<sup>20-22</sup> and should be guarded against. Critical examination for the presence of such biases in sensitivity and funnel plot analyses is therefore advised and should form an integral part of meta-analyses, as appropriate.<sup>23,24</sup>

### **1.2.1.3 Step 3: Quality assessment of studies**

Quality assessment of individual studies that are summarized in systematic reviews is necessary to limit bias in conducting the review, gain insight into potential comparisons, and guide interpretation of findings. Factors that warrant assessment are those related to applicability of findings (also called external validity or generalizability), validity of individual studies, and certain design characteristics that affect interpretation of results.<sup>11</sup> Assessment of study quality is relevant to every step of a systematic review.

Internal validity is the extent to which the design and conduct of a study are likely to have prevented systematic error (bias). In studies of the effects of health care, the main types of bias arise from systematic differences in the groups that are compared (selection bias), the care that is provided, exposure to other factors apart from the intervention of interest (performance bias), withdrawals or exclusions of people entered into a study (attrition bias) or how outcomes are assessed (detection bias).<sup>11,25</sup>

External validity is the extent to which results provide a correct basis for generalizations to other circumstances (including patient characteristics, treatment regimens, settings and modalities of outcomes).<sup>11,25</sup>

Study design is extremely important and determines the validity of the observed effects, i.e. our confidence that the results of a study are likely to approximate to the "truth" for the participants or patients studied depends on the soundness of its design.<sup>18</sup> In this way design serves as a marker of study quality. Ultimately the strength of a review's conclusions depends on the integrity of designs of the available studies. It is important to note that different types of questions may require the use of different study designs. A hierarchy of study designs have been proposed by many authors

and groups<sup>18,26-28</sup> and can serve as an indication of the level of evidence based on soundness of design (Table 1.3).

**Table 1.3: A hierarchy of study designs: levels of evidence and grades of recommendation\***

Study design	Level of evidence based on soundness of design	Grades of recommendation
<ul style="list-style-type: none"> <li>• Meta-analysis of Randomized Controlled Trials (RCT)</li> <li>• RCT – with concealed allocation</li> </ul>	<b>I</b>	<b>A</b>
<ul style="list-style-type: none"> <li>• Experimental study without randomization (quasi-experimental / quasi-randomized / pseudo-randomized studies)</li> <li>• Cohort studies</li> <li>• Case-control studies</li> </ul>	<b>II</b>	<b>B</b>
<ul style="list-style-type: none"> <li>• Cross-sectional studies</li> <li>• Before-and-after studies / Comparative studies</li> <li>• Case series</li> </ul>	<b>III</b>	
<ul style="list-style-type: none"> <li>• Case reports</li> <li>• Pathophysiological studies or bench research</li> <li>• Expert opinion or consensus</li> </ul>	<b>IV</b>	<b>C</b>

\*Source: Adapted from references<sup>18,26,27,28</sup>

Moher et al.<sup>29,30</sup> identified numerous scales and checklists that have been used to assess the validity and quality of trials, all with reported limitations. Therefore the Cochrane Collaboration has advised that none of the currently available scales for measuring the validity or quality of trials can be recommended without reservation.<sup>11</sup> This group recommends that it is preferable to use simple approaches for assessing validity that can be fully reported. The Collaboration's recommended tool for assessing risk of bias is neither a scale nor a checklist. It is a domain-based evaluation (Cochrane "risk of bias" tool), in which critical assessments are made separately for different domains, i.e. sequence generation, allocation concealment, blinding, incomplete outcome data, selective outcome reporting and other sources of bias.<sup>11</sup>

#### **1.2.1.4 Step 4: Collection / Extraction of data; Summarizing the evidence; Analysing and presenting results**

The data collection/extraction form is the link between the primary source (e.g. individual journal articles) and what is ultimately reported by the reviewer. Sufficient time, thought and planning should thus be invested in the design of these forms. Key components of these forms include information regarding study references and reviewers, verification of study eligibility and study characteristics (including methods, participants, interventions and outcomes).<sup>11</sup> The data collection form should be designed bearing in mind the specific review questions and planned analyses at all times.<sup>7,11</sup> Blinded data extraction (i.e. to the authors, journal and results when assessing quality) has been proposed. Although there is some evidence that blinded assessments of trial quality may be more reliable and different from assessments that are not blinded,<sup>31,32</sup> blinding is difficult to achieve, time consuming and may not substantially alter the results of a review.<sup>33</sup>

Data synthesis involves collating and summarizing the results of the included primary studies. Synthesis can be narrative/descriptive (non-quantitative).<sup>17</sup> Narrative synthesis uses subjective (rather than statistical) methods for reviews where meta-analysis is either not feasible or not sensible. In the case of narrative reviews, care should be taken to not introduce bias by inappropriately stressing the results of one/some studies over others.<sup>11</sup> It is sometimes possible to complement a descriptive synthesis with a quantitative summary. Using statistical techniques to obtain a quantitative synthesis is referred to as meta-analysis, and results are often displayed graphically (typically using forest- and funnel plots).<sup>17,34</sup> The value a meta-analysis can add to a review depends on the context in which it is used, and reasons for possibly including a meta-analysis in a review include to increase power, to improve precision, to answer questions not posed by the individual studies and to settle controversies arising from apparently conflicting studies or to generate new hypotheses.<sup>11</sup> Well conducted meta-analyses allow an objective appraisal of the evidence, provide a more precise estimate of treatment effect, and may explain heterogeneity between the results of individual studies.<sup>35</sup> Opinions will often differ on the correct method for performing a particular meta-analysis. In this regard, the robustness of the findings to different assumptions about the data and the methods that were used can be examined in a thorough sensitivity analysis.<sup>11,35</sup>

### **1.2.1.5 Step 5: Interpreting the results / findings**

It can be argued that the results of a systematic review/meta-analysis should stand on their own, but in fact many people turn to the Discussion and Conclusions for help with interpreting the results. Discussion and conclusions about the following issues can help people to make decisions:<sup>11</sup>

- The strength of the evidence
- The applicability of the results
- Other information (e.g. considerations of costs and current practice)
- Clarification of any important trade-offs between the expected benefits, harms and costs of the intervention

This section of the systematic review should be able to help people to understand the implications of the evidence in relation to practical decisions.<sup>11,17</sup> It should be considered that the purpose of a systematic review is to present information, rather than to offer advice. Important limitations of the systematic review should also be highlighted here and placed in context when interpreting the findings.<sup>11,17</sup>

### **1.2.2 Limitations of Systematic Reviews and Meta-analyses**

The limitations and possible problems associated with systematic reviews and meta-analyses have been recognised and are described in the literature.<sup>11,24,25,34,36</sup> Some of these problems may have their roots in the poor quality of the original studies, while others may be related to statistical inference.<sup>34</sup> Special care should be taken in the selection of studies to include in a meta-analysis, using well-defined strategies to overcome publication and reviewer (observer) bias.<sup>34</sup> Biases in publication, location [including English language bias (especially), database bias, citation bias, multiple publication bias and bias in provision of data], and inclusion are potentially serious problems in systematic reviews and meta-analyses and should be guarded against and accounted for as far as possible.<sup>24</sup> Critical examination for the presence of such biases (through sensitivity and funnel plot analyses) should form part of meta-analyses as appropriate.<sup>24</sup> Disadvantages and criticisms of systematic reviews have been described and include: synthesis of results may disguise or oversimplify important distinctions between primary studies with regard to inclusion/exclusion criteria or the nature of an intervention, reviews of similar topics may appear to reach different conclusions depending on the precise form of the "review question", reviews may

make it difficult for practitioners to apply the results of studies to the specific characteristics of the situation in which they find themselves (over-generalization) and the findings from systematic reviews are not always consistent with the findings of large-scale high quality trials.<sup>36</sup> It is important to keep in mind that a meta-analysis is only as good as its components, i.e. the trials (and specifically the study quality of the trials) that make up the various outcomes. Systematic and explicit descriptions and methods of making judgements and reaching conclusions can reduce errors and greatly improve the precision of the systematic review. Systematic reviews can aid, but never replace, sound clinical reasoning. Evidence can lead to poor practice if it is applied in an uncritical way. Understanding the complex structure of decision making requires an appreciation of the ways in which knowledge, skills, values and research evidence are integrated in each patient-clinician encounter.<sup>10</sup>

### **1.2.3 Conclusions: Evidence-based Nutrition**

Evidence-based healthcare is new in that it is more explicit and systematic in the collection and application of evidence, and places more emphasis on empirical evidence. It complements and does not replace experience, judgement and caring. With rare exceptions, no study, whatever the type, should be interpreted in isolation. Systematic reviews are required of the best available type of study for answering the clinical question posed.<sup>37</sup> A criticism of systematic reviews that have been voiced is that they are often unable to provide specific guidance on effective (or even ineffective) interventions. Instead, they often conclude that little evidence exists to allow the question to be answered (often because the primary studies that they include contain few outcome evaluations).<sup>38</sup> This in itself, however, remains an important contribution; it is, after all, only through mapping what is known and acknowledging uncertainty that scientific knowledge can accumulate.<sup>38</sup> Well-conducted systematic reviews still provide us with the best available evidence.

## **1.3 MICRONUTRIENTS IN THE CRITICALLY ILL PATIENT**

Nutritional support of the critically ill patient includes the daily provision of vitamins and trace elements. These compounds, collectively termed “micronutrients,” are essential not only as intermediaries in metabolism but also for their potential roles in cellular immunity, wound healing and antioxidant activity.<sup>39</sup> Micronutrient deficiencies in critically ill patients may occur as pre-existing conditions in patients

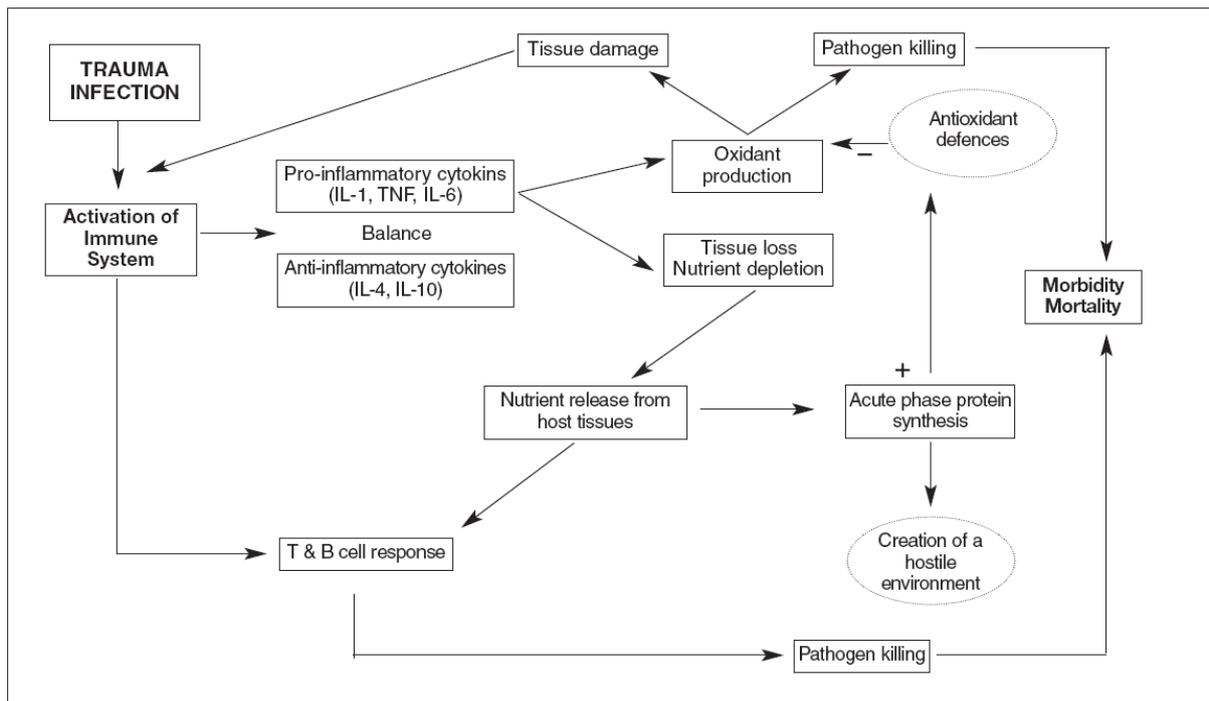
with poor nutritional status prior to hospitalization or as a result of severe illness or the injury itself.<sup>5</sup> Any injured patient will develop an acute phase response (APR) and a systemic inflammatory response syndrome (SIRS) with the production of various mediators, including cytokines, which modulate the metabolic response.<sup>40,41</sup> SIRS is associated with a redistribution of vitamins and trace elements from the circulating compartment to tissues and organs, which are involved in protein synthesis and immune cell production.<sup>42</sup> The circulating concentrations of most trace elements (iron, selenium, zinc) and of their carrier proteins decrease as do the water-soluble vitamins, whereas copper and manganese increase,<sup>42,43</sup> causing a relative deficit in circulating antioxidants. In addition, trauma and burns patients typically experience extensive losses of biologic fluids through wound exudates, drains and haemorrhage, which contribute to negative micronutrient balances.<sup>41</sup> These deficient states can affect various biochemical processes and enzymatic functions, resulting in organ dysfunction, poor wound healing and altered immune status – all with deleterious patient outcomes.<sup>5</sup> Therefore attention to micronutrient requirements in the critically ill is imperative. This brief overview will outline the metabolic response to stress and the interrelated effects of the APR on micronutrient status so as to provide a rational basis for specialized nutritional support and specifically micronutrient supplementation.

### **1.3.1 Metabolic Response to Stress**

Following trauma or other tissue injury the host initiates a cascade of metabolic-, hormonal- and mediator-induced reactions in an effort to prevent ongoing tissue damage and to facilitate the repair of damaged tissue as well as to restore tissue function to normal.<sup>44</sup> The immediate sets of reactions that are so induced are collectively known as the acute phase response (APR) first described by Cuthbertson more than 70 years ago,<sup>45</sup> who later divided the response to injury into the well-known ebb and flow phases.<sup>46</sup> The former is characterized by a state of hypovolaemia, shock and tissue hypoxia and the latter by hypermetabolism.

The neuro-endocrine response to trauma results in an exaggerated mobilisation of metabolic substrates and a loss of the adaptive decrease in Resting Energy Expenditure (REE) and nitrogen excretion seen in starvation. In contrast to the substrate dependency of uncomplicated starvation, trauma and injury are neuro-

endocrine-driven processes.<sup>47</sup> The hormonal response favours endogenous sources of fuel as its primary energy source<sup>48</sup> and produces a rapid breakdown of body protein and a rapid and maximal rate of fat oxidation. Additionally, the two, principal, proximal cytokines, interleukin 1 (IL-1) and tumor necrosis factor (TNF- $\alpha$ ), both of which stimulate the production of a third, pivotal cytokine, interleukin 6 (IL-6) are released by macrophages after an insult to the host<sup>49</sup> and are described as pro-inflammatory cytokines as they are key mediators of inflammation.<sup>50</sup> Many of the signs and symptoms experienced during infection and following injury and surgery, such as fever, loss of appetite, weight loss, negative nitrogen and micronutrient balance and lethargy, are caused directly and indirectly by pro-inflammatory cytokines. Indirect effects of cytokines are mediated by actions upon the pituitary and adrenal glands and endocrine pancreas, resulting in increased secretion of the catabolic hormones adrenalin, nor-adrenalin, glucocorticoids and glucagons.<sup>51</sup> Studies, investigating the metabolic effects of interferon-gamma (IFN- $\gamma$ ), suggest that IFN- $\gamma$  might also be playing a major role in the metabolic response to infection and injury.<sup>52</sup> Cytokines are also known to be responsible for the alterations seen in energy expenditure, gluconeogenesis, lipolysis, vascular permeability and skeletal muscle proteolysis.<sup>53</sup> Additionally, the increased hepatic synthesis of acute phase proteins is triggered by the release of cytokines.<sup>53</sup> The production of cytokines is part of the highly effective mechanism for creating a hostile environment for pathogens within the body,<sup>54</sup> while promoting tissue repair. The key features of the inflammatory response are displayed in Figure 1.1.



**Figure 1.1: Key features of the inflammatory response (from reference<sup>55</sup>)**

+: a stimulatory effect; -: an inhibitory effect

This systemic inflammatory response syndrome (SIRS) is clinically identified by generally agreed upon characteristics of elevation or decrease in white blood cell counts, increase in heart rate, rise or fall in body temperature and changes in respiratory rate or arterial oxygen concentration when breathing room air.<sup>56</sup> In the vast majority of instances the various effects of the SIRS lead to an improved outcome from injury, infection or inflammation of limited duration in the previously well-nourished host. Balancing this pro-inflammatory cascade is the so-called compensatory anti-inflammatory response syndrome (CARS).<sup>57</sup> It has been hypothesized, from a growing body of research data, that the relative balance between SIRS and CARS has an impact on survival.

In nutritional terms, trauma, for instance, profoundly alters energy metabolism with the characteristic elevation of REE.<sup>58</sup> The metabolic response to trauma also involves an increased loss of body proteins due to accelerated protein degradation, increased amino acid catabolism and nitrogen loss. In the liver, the rate of synthesis of selected proteins [i.e. albumin, transferrin, prealbumin, retinol binding protein (RBP) and fibronectin] is decreased, whereas acute phase protein synthesis is increased.<sup>59</sup> Carbohydrate metabolism during the APR is characterised by various degrees of

hyperglycaemia,<sup>60</sup> decreased glucose tolerance and insulin resistance.<sup>49,61</sup> These characteristics are the result of increased glycogenolysis<sup>49,62</sup> and non-suppressible gluconeogenesis from substrates mobilised peripherally.<sup>49,62,63</sup> The findings in lipid metabolism following trauma involve several complex changes in the mobilisation and oxidation of fat. Studies of lipid metabolism have not always yielded a consistent picture with variable changes in plasma levels of triglycerides, free fatty acids (FFA), ketone bodies and very low density lipoproteins (VLDL) being reported.<sup>64</sup>

### **1.3.2 Effect of the Acute Phase Response on Micronutrient Status**

Despite the relatively well described changes in macronutrient metabolism in the presence of the APR, the understanding of both the changes in micronutrient metabolism and implications thereof in terms of clinical outcomes remain less well defined.

The increased metabolic rate and catabolism associated with the APR are thought to increase the requirements of such micronutrients as vitamin A, E, B<sub>6</sub>, C, D and folate.<sup>65-67</sup> Serum levels of various vitamins decrease with the inflammatory response, though the clinical significance of this remain uncertain.<sup>5,43</sup> For example levels of vitamin A, C and E are decreased in postoperative patients<sup>65,68</sup> and septic patients have high vitamin A excretion in the urine.<sup>69</sup> On the other hand it is also known that a redistribution in plasma trace elements occurs with a decrease in the concentrations of Iron (Fe), Zinc (Zn) and Selenium (Se) and a concomitant rise in Copper (Cu) levels.<sup>64,70-72</sup> In this regard, it is generally accepted that increased needs due to increased losses are often compounded by decreased intake, especially in surgical patients.

The interpretation of low plasma levels observed in critically ill patients is complex and remains problematic, as the causes are multi-factorial. SIRS redistribution play an important role together with acute losses through biological fluids (exudates,<sup>42</sup> drains,<sup>73</sup> effluents from continuous renal replacement,<sup>74</sup> chylous losses,<sup>75</sup> other digestive losses), dilution as a result of resuscitation fluids and insufficient intakes also contribute.

It is thus clear that highly coordinated changes take place in the plasma concentration of vitamins and trace elements after trauma or during illness. In order to provide an optimal micronutrient intake that is both adequate and safe, it is necessary to understand their metabolism and various mechanisms of action in the context of the APR.

### **1.3.2.1 Mechanisms and benefits of changes in trace element concentrations**

#### **1.3.2.1.1 Iron**

Serum Fe concentration have been documented to decrease during infections,<sup>76,77</sup> after an endotoxin injection<sup>78</sup> as well as after cytokine administration.<sup>77-79</sup> The plasma concentration of Fe falls rapidly after injury, a significant fall being evident  $\pm$  2-4 hours after a skin incision. The plasma concentration continues to fall until 12-24 hours after the beginning of surgery. Thereafter, plasma Fe tends to remain low for several days.<sup>80</sup> Van Iperen et al.<sup>81</sup> reported that serum Fe concentrations dropped to 23 and 46% preoperative levels after major and minor surgery respectively and remained low for up to 28 days after major surgery. Serum transferrin concentrations and transferrin saturation decreased after both types of surgery while ferritin concentrations increased. Serum transferrin receptor concentrations increased only four weeks after major surgery.

The fall in plasma Fe concentration is thought to arise from the transfer of Fe from the Fe-transferrin complex in the plasma to other proteins. Initially, Fe is transferred to lactoferrin, released from leukocytes at the sites of inflammation. Fe is then taken up and bound to ferritin in the liver and spleen.<sup>64,82</sup> Depletion of Fe from serum is thus associated with an increased Fe and ferritin content in the liver. Cytokines, generated by activated T cells and macrophages orchestrate the removal of iron from the plasma.<sup>83</sup> The cytokines IL-1, IL-6 and TNF are accepted as being the major mediators altering Fe dynamics.<sup>84,85</sup> Even minor tissue damage can initiate an APR, including the release of cytokines, and is sufficient to produce changes in iron metabolism. The main cause of anaemia at this time is increased clearance of Fe into non-hemopoietic tissues.<sup>86</sup>

The movement of Fe into a storage form reduces its availability within the plasma, thereby withholding Fe from bacteria<sup>87-89</sup> and reducing the conversion of superoxide

radicals to free hydroxyl radicals, thus reducing oxidative damage to membranes or DNA.<sup>80</sup> The hypoferraemic response that develops during the APR, in relation to trauma or infection, is thus thought to be a protective response<sup>90-95</sup> that aids the host by decreasing the availability of iron for use by invading micro-organisms.

Should the above-mentioned changes in Fe dynamics persist, Fe transfer to bone marrow is reduced, leading to the anaemia of chronic disease,<sup>83,96</sup> and in the long term, potential Fe depletion. The degree of "anaemia of chronic disease" is directly related to the extent of injury.<sup>81</sup>

#### **1.3.2.1.2 Zinc**

An initial increase in serum Zn concentrations and in the zinc:albumin ratio are seen after trauma or surgery, followed by a decrease in both parameters.<sup>80</sup> This decrease in serum levels of Zn that occurs in response to physiologic stress has been well documented<sup>97-102</sup> and may be the result of increased losses, dilution and/or redistribution in the presence of the APR.<sup>103</sup>

The initial increase in Zn concentrations may be explained by the release of Zn from intracellular stores, in response to tissue damage.<sup>80</sup> The subsequent decrease in serum Zn is related to the fall in serum albumin concentrations seen in all seriously ill patients as a result of increased transcapillary escape to the extravascular compartment.<sup>101</sup> As most plasma Zn circulates bound to albumin, a significant fall in plasma albumin leads to a fall in Zn concentration. During the APR the drop in plasma Zn is usually greater than that in albumin, indicating the active transfer of Zn from its albumin binding site to some other body compartment. The removal of Zn from its main transport protein is thought to be mediated by IL-1.<sup>71,78</sup>

The decrease in serum Zn may also be related to a redistribution of Zn to the site of tissue injury as studies have shown that Zn localises in actively healing wounds.<sup>104</sup> Zn can also decrease in serum as a non-specific reaction to stress. Surgical trauma increases corticosteroid secretion, which decrease the serum Zn concentration.<sup>105</sup> Hypermetabolic trauma patients may experience excessive gastrointestinal Zn losses.<sup>106</sup> The urinary excretion may also contribute to the low plasma concentration in these patients.<sup>107</sup> Additionally, Berger et al.<sup>108</sup> demonstrated high cutaneous Zn

losses in thermal injury patients. Another mechanism seems to be a combination of the induction of the metal binding protein metallothionein in the liver,<sup>109</sup> and increased uptake of zinc into the liver to bind to metallothionein.<sup>110</sup>

Several reasons have been proposed why lowering of serum Zn levels with liver accumulation should benefit the host. These include repair of damaged tissue, protection of the liver from endotoxins, co-factor function in acute phase protein synthesis and increased bacteriocidal capability after phagocytosis.<sup>49</sup> Zn is also an essential part of the Zn-finger components of the DNA-binding transcription factors, which are important in controlling the selectivity of protein synthesis.<sup>82</sup> Alterations in Zn levels are also thought to improve host defense mechanisms by prevention of microbial proliferation.<sup>111</sup>

#### **1.3.2.1.3 Selenium**

In both acute<sup>112-114</sup> and chronic<sup>115</sup> illnesses, the plasma concentration of Se decreases in proportion to the magnitude of the inflammatory response.

Se concentrations follow a similar pattern to the described changes in serum plasma trace element concentrations in the presence of the acute phase reactants. Se concentrations decrease by 10% at day 1 following minor surgery, followed by a subsequent increase toward the starting concentration by day 6.<sup>116</sup>

Low plasma Se concentrations have been described in a variety of clinical conditions such as myocardial infarction, severe burns,<sup>117</sup> acute pancreatitis and in intensive care patients.<sup>112</sup> Low plasma Se concentrations have also been described in chronic conditions such as cancer.<sup>115</sup> Studies done in thermal injury<sup>117</sup> have demonstrated decreased serum Se concentrations, essentially throughout the hospital course, with slightly elevated urinary losses on days 3-7 post-injury. In relation to selenium function, Hunt et al.<sup>118</sup> demonstrated decreased erythrocyte glutathione peroxidase activity in thermal injury, which would support the concept of true Se deficiency rather than a merely decrease in its carrier proteins.

It has been proposed that the aetiology for the observed decrease in serum Se levels in thermal injury is multifactorial and may include a manifestation of the APR,

drug toxicity (through oxidative stress mechanisms),<sup>119</sup> a decreased red blood cell survival rate that is often observed in thermal injury<sup>120</sup> and/or an antagonistic relationship with silver.<sup>117,121</sup>

It has also been proposed that selenium concentration falls largely due to increased capillary escape of selenoprotein P, the major selenium containing protein in the plasma.<sup>122</sup> The benefit of this is probably to deliver selenium and antioxidant activity to the interstitial fluid, as well as to the other tissues of the body. It has been speculated that the translocation of selenium may be important in activating nuclear factor-kappa B (NF-κB), leading to increased acute phase protein synthesis.<sup>123</sup> It is interesting to note that the more severe the illness, the lower the selenium concentrations.<sup>124</sup> Mishra et al. were able to demonstrate that the greater the organ damage as measured by the Sequential Organ Failure Assessment (SOFA) score, the lower the plasma selenium concentrations.<sup>125</sup>

### **1.3.2.2 Changes in plasma concentrations of vitamins during the APR**

The fall in concentrations of most trace elements is mirrored by falls in many blood vitamins. This would appear to be partly as a result of transcapillary escape of carrier proteins, and partly as a result of movement intracellularly to catalyze the increase in metabolic rate and also to provide increased antioxidant activity.<sup>65,126</sup>

Longitudinal studies<sup>65</sup> in the presence of the APR have demonstrated a transient, but significant, decrease in the concentrations of leukocyte vitamin C, and in plasma concentrations of vitamin A, retinol-binding protein (RBP), vitamin E, total lipids, pyridoxal-5'-phosphate and albumin during the APR. Blood concentrations of pyridoxal-5'-phosphate, RBP and leukocyte vitamin C decreased to values below the respective normal ranges but normalized without any therapeutic interventions upon the resolution of the APR. The transient and self-correcting nature of the decreased values argues against a true deficiency state, although it must be noted that the APR in this study population was of a short duration.

In terms of antioxidant defences, antioxidant status and lipid peroxidation were studied by Goode et al<sup>127</sup> in patients with sepsis and multisystem organ failure. Plasma concentrations of α-tocopherol were significantly lower than in healthy control

subjects, whilst beta carotene and lycopene concentrations were decreased below the respective reference ranges. Borelli et al.<sup>128</sup> measured daily plasma concentrations of ascorbic acid and  $\alpha$ -tocopherol as predictors of multisystem organ failure in intensive-care patients. Plasma ascorbic acid concentrations were significantly lower in the patients that developed multisystem organ failure, although  $\alpha$ -tocopherol levels were not significantly different. Antioxidant vitamin status has also been studied in surgical patients. Agarwal et al.<sup>68</sup> studied the serum concentrations of ascorbic acid and the tocopherols in 57 surgical patients. All vitamin concentrations decreased significantly on day 1 after surgery, with a maximal decrease by day 3 post-surgery (41% in ascorbic acid, 27% in  $\alpha$ -tocopherol, 31% in  $\gamma$ -tocopherol). Vitamin concentrations had returned to normal by day 7 after the operation. A subgroup of patients with postoperative infections had significantly lower preoperative concentrations of  $\gamma$ -tocopherol. Lower preoperative concentrations of  $\alpha$ -tocopherol and  $\gamma$ -tocopherol were significantly correlated to mortality in the 6 patients who died during the course of the study.

So in summary, a large number of highly complex coordinated mechanisms have evolved to move micronutrients around the body, and to ensure that they are in the correct place and in the correct concentration. This integrated mechanism is clear evidence of the importance of micronutrients in critical illness.<sup>126</sup>

### **1.3.3 Micronutrients and the Oxidative Stress State**

Oxidative stress has been implicated in the manifestations of critical illnesses, including ischemia and reperfusion injury and systemic inflammatory states.<sup>129</sup> It is increasingly being recognized as vital to the underlying pathophysiology of critical illness, particularly the development of organ failure.<sup>130</sup> Oxidative stress is defined as “a state in which the level of toxic reactive oxygen intermediates (ROI) / reactive oxygen species (ROS) overcomes the endogenous antioxidant defenses of the host”.<sup>129</sup> Oxidative stress can result from either an excess in oxidant production, and/or depletion of antioxidant defenses.<sup>131,132</sup> Protective antioxidant systems help defend against ROS induced cellular damage. Oxidative stress is thought to increase in the presence of the APR because of the accompanying activation of neutrophils and macrophages and the subsequent release of free radicals,<sup>133</sup> which may overwhelm defensive mechanisms and disturb the pro-oxidant-antioxidant balance

in favour of the former, leading to potential damage.<sup>134</sup> Besides ROS, another category of free radicals is derived from nitric oxide (NO) metabolism and is the normal byproduct of endothelial metabolism.<sup>42</sup> Free radicals cause a cascade of intracellular events resulting in the release of NF- $\kappa$ B in the cytoplasm,<sup>135</sup> and subsequently enabling the initiation of the transcription process. NF- $\kappa$ B controls the production of acute phase mediators such as TNF- $\alpha$ , IL-2, and IL-2 receptors, which in turn activate NF- $\kappa$ B, intensifying the inflammatory cascade.<sup>42</sup> In this regard, selenium has been shown to downregulate NF- $\kappa$ B, thus limiting the extent of the APR<sup>136,137</sup> whereas antioxidants in general are considered to limit the release of NF- $\kappa$ B caused by ROS.<sup>138</sup> Oxidative stress can cause lipid peroxidation, damage to DNA, and cell death<sup>139</sup> and has been associated with sepsis, shock, mechanical ventilation, organ dysfunction, adult respiratory distress syndrome (ARDS) and surgery.<sup>134</sup>

Thus, critical illness is associated with increased ROS production (and thus increased oxidative stress), and on the other hand low levels of most antioxidant micronutrients (endogenous antioxidant defenses). Good and reliable methods of assessing overall antioxidant status are therefore important, but remain problematic. Measurement of individual micronutrients in the plasma is of limited value, whilst the measurements of total antioxidant capacity (TAC), though promising, have not yet been shown to have sufficient sensitivity or specificity for clinical use.<sup>140</sup> Proof of an increased oxidative stress state is difficult to obtain based solely on measured levels of ROS, due to their short half-life.<sup>132</sup> Evidence for an increased oxidative stress state in critically ill patients is seen indirectly through the measurement of the byproducts of ROS with cellular molecules. These patients demonstrate elevated levels of substances such as thiobarbituric acid-reacting substances (TBARS: byproducts of the interactions of lipids with ROS), DNA and proteins.<sup>5</sup> Oxidative damage markers, such as malondialdehyde (MDA) or F2-isoprostanes, reflect the balance between oxidant stress and the body's ability to cope with it,<sup>141</sup> and are likely to be more useful than antioxidant protection markers in predicting outcome.<sup>142</sup>

Endogenous mechanisms work in a network-like fashion to neutralize the production of ROS in an attempt to counteract the deleterious effects thereof.<sup>5</sup> Intracellular glutathione and nonenzymatic ROS scavengers (including vitamins such as ascorbic acid,  $\beta$ -carotene and  $\alpha$ -tocopherol) form part of this highly evolved mechanism.

Enzymatic systems [including superoxide dismutase (SOD), catalase and glutathione peroxidases (GSHPx)] then work synergistically to detoxify ROS further. These enzyme systems are dependant on minerals such as selenium, copper, zinc and manganese as important cofactors in these enzymatic reactions.<sup>5</sup>

Of all the antioxidants, special interest is currently being afforded to selenium as a result of recent supplementation trials. Selenium-dependant enzymes and selenoprotein P regulate immune and endothelial function. Four of the six known glutathione peroxidases play a significant role in antioxidant defenses.<sup>42</sup> The mechanisms involved have recently been reviewed<sup>143</sup> in detail and indicate that it is not necessarily selenium itself but rather the activity of selenium-dependant enzymes that are of crucial importance.

#### **1.3.4 Micronutrient Supplementation in Critical Illness**

Research on micronutrient supplementation in the critically ill has focused mainly on five micronutrients: selenium, zinc, copper, vitamins C and E, and more recently also the vitamin B group.<sup>2,42</sup> The aims of supplementation can be described as provision of basic nutritional support (bearing in mind the increased requirements due to hypermetabolism and wound healing), prevention and correction of deficiencies, and modulation of the APR and immune responses by reinforcement of endogenous antioxidant defences.<sup>2</sup> Establishing requirements in the critically ill has proven notoriously difficult and various sets of guidelines (each with their own limitations in this population) and proposals from various authors are available (Table 1.4; for the five mentioned micronutrients).

**Table 1.4: Recommended doses of selected micronutrients in critical illness**

Micro-nutrient (MN)	RDA for oral feeding (daily) <sup>144,145</sup>	Recommendations for PN			Proposed supplements (in addition to MNs provided by feeding)		
		AMA 1979 <sup>146,147</sup>	Shenkin 1995 <sup>148</sup>	FDA 2000 <sup>149</sup>	Berger 2006 <sup>41</sup>		Fuhrman 2002 (/d) <sup>150</sup>
					Major trauma (5d)	Major burns (14-21d)	
Vitamin C (mg)	60	100	100	200	1000 (IV)	1000 (IV)	500 – 3000
Vitamin E (mg)	8 – 10	10	10	10	100 (EN)	100 (EN)	400 (IV) 40 – 1000 (EN)
Selenium (µg)	55 – 70	30 – 60	60	-	300 (IV)	500 (IV)	100 – 400
Zinc (mg)	12 – 15	2.5 – 4	6.5	-	20 (IV)	30 (IV)	10 – 30
Copper (mg)	2	0.5 – 1.5	1.3	-	-	4 (IV)	-

AMA: American Medical Association; d: day; EN: Enteral nutrition; FDA: Food and Drug Administration; IV: Intravenous; MN: Micronutrient; PN: Parenteral nutrition; RDA: Recommended dietary allowance

Despite these guidelines and proposals little consensus exist as to “what to use when” and many questions remain regarding doses required, route and timing of replacement. It is also imperative to bear in the mind the potential for deleterious effects, i.e. “more is not necessarily better”. Micronutrients, especially trace elements and fat soluble vitamins, carry the risk of toxicity at high intake levels.<sup>41,42</sup> Nutrition support practitioners considering supplementing antioxidant micronutrients in critically ill patients should proceed with caution and consider the amounts of such micronutrients a patient is already receiving through an oral diet and/or enteral/parenteral nutritional support. Inappropriate antioxidant micronutrient supplementation have the potential for creating a pro-oxidant microenvironment that may have as much potential for harm as for benefit in the well-nourished patient.

A growing body of evidence is emerging that demonstrate the potential benefits of micronutrient (and specifically antioxidant) supplementation in critically ill patients. These micronutrient supplementation trials form the basis of this systematic review and will be taken up in detail in the results and discussion sections.

### **1.3.5 Conclusions: Micronutrients in the Critically Ill**

There has been growing interest in micronutrients as a result of their essential role in endogenous antioxidant defence mechanisms and immunity. Critically ill patients are characterized by increased free radical production and thus an increased oxidative stress state, depending on the severity of illness. In addition, SIRS is associated with a redistribution of micronutrients and patients typically experience extensive losses of biologic fluids which further contribute to negative micronutrient balances and subsequently an imbalance in endogenous antioxidant capacity. The interpretation of low plasma levels observed in critically ill patients is complex and remains problematic, with oxidative damage markers, such as MDA or F2-isoprostanes showing promise in predicting outcome. Micronutrient supplementation in trauma and burns patients seems beneficial by controlling the oxidative stress caused by the acute condition. Further research is imperative so that selective micronutrient therapy can be initiated as early and safely as possible in all critically ill patients.

### **1.4 MOTIVATION FOR THE STUDY**

Micronutrient studies in the critically ill remain few in relative terms, with problems arising from heterogeneity of patient populations, large variability of patients within the same diagnostic category and absence of relevant clinical endpoints. Nevertheless, emerging evidence regarding the potential of micronutrient supplements in influencing clinical outcomes in the critically ill is encouraging.

Despite two published systematic reviews already available in the literature,<sup>130,151</sup> the sum-total of available evidence (including the outcome of these two meta-analyses) still indicate that the exact micronutrient requirements of the critically ill patient and related practice issues remain uncertain, complicating decision-making on the part of the nutrition support practitioner. Since the systematic review by Heyland et al.<sup>130</sup> was published in 2005 (wherein they searched the literature until December 2003), several trials were conducted (including larger multi-centre trials) prompting the investigator to explore the questions once more by aggregating the latest trial data and results with those of the older trials, with the aim of obtaining better clarity and further answers. The recently updated Cochrane systematic review by Avenell et al.<sup>151</sup> focused on selenium supplementation (only) in critically ill adults, and as such,

although selenium was included in this review, a sub-group analysis of selenium vs. non-selenium containing micronutrient supplements was not undertaken.

This systematic review therefore evaluated the latest evidence that is available regarding micronutrient supplementation and clinical outcome in the critically ill patient. The findings will help facilitate the implementation of up-to-date guidelines for nutrition support practitioners with regard to the use of micronutrient supplementation in this population group, until even further evidence becomes available.

## **CHAPTER 2: METHODOLOGY**

## **2.1 STUDY OBJECTIVES**

### **2.1.1 Purpose of the Study**

This systematic review assessed the effects of micronutrient supplementation\* on adults recovering from critical illness†.

### **2.1.2 Specific Objectives**

The primary objectives of the review were to examine the effect of micronutrient supplementation in critically ill adult patients on:

- mortality
- number of infectious complications
- length of intensive care unit stay (LICU)
- length of hospital stay (LOS)

(Meta-analysis)

The secondary objectives were to *briefly describe* (in relation to micronutrient supplementation):

- the timing of micronutrient support, the doses administered and practice issues as reported in the various studies
- the micronutrient status of the patients
- morbidity
- the course of the APR as judged by cytokine levels, White Blood Cells (WBC) and differential WBC count, and other inflammatory markers [specifically C-reactive protein (CRP)]
- the changes observed in the level of oxidative stress (as measured in the various studies)

(Descriptive)

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\* For the purpose of this systematic review "micronutrient supplementation" refers to the administration of micronutrients (enterally and/or parenterally) over and above the micronutrients already included in the enteral / parenteral formula(s) the patient is receiving as part of normal nutritional support.

† For the purpose of this systematic review "critically ill patients" includes medical, surgical and burns patients treated in an Intensive Care Unit (ICU).

## **2.2 CRITERIA FOR CONSIDERING STUDIES FOR THIS REVIEW**

### **2.2.1 Types of Studies**

All randomized controlled trials (RCTs) of micronutrient supplementation in critically ill patients, given in addition to their routine care, were included. Trials were included despite lack of blinding or placebo treatment.

### **2.2.2 Types of Participants**

All studies in human adults with critical illness were included. Studies on neonates and paediatric patients (<14 years) were excluded.

### **2.2.3 Types of Intervention**

Nutritional interventions by the enteral and/or parenteral route, supplemented with additional micronutrients versus nutritional care via the same route without additional micronutrients were examined. Immunonutritional interventions where the micronutrients were part of multiple nutrients given (for example with arginine and omega-3 fatty acids) were excluded.

### **2.2.4 Types of Outcome Measures**

Information was sought on the following primary outcomes:

- mortality
- number of infectious complications
- length of intensive care unit stay (LICU)
- length of hospital stay (LOS)

Additionally, information was sought on:

- timing of micronutrient support
- micronutrient doses administered
- micronutrient status
- morbidity
- course of the APR by
  - cytokine levels
  - WBC and differential WBC count
  - inflammatory markers (specifically CRP)
- level of oxidative stress

## **2.3 SEARCH METHODS FOR IDENTIFICATION OF STUDIES**

To locate articles to be included in this systematic review, several electronic bibliographic databases were searched from inception up until 29 February 2008. To establish the extent to which this topic has been researched and to allow for all relevant studies to be included, the search period was not restricted. In addition, bibliographies of retrieved articles were reviewed and personal (private) files searched to obtain additional citations.

### **2.3.1 Data Sources**

An electronic search was conducted and the following major databases were searched to identify potential relevant citations:

- Pubmed – MEDLINE [1950 – 29 February 2008]
- Science Direct [1823 – 29 February 2008]
- ISI Web of Knowledge – Web of Science (Science Citation Index Expanded / SCI-EXPANDED) [1987 – 29 February 2008]
- Cochrane Library [1800 – 29 February 2008], including:
  - Cochrane Database of Systematic Reviews (CDSR; Cochrane Reviews)
  - Cochrane Central Register of Controlled Trials (CENTRAL; Clinical Trials)
  - Database of Abstracts of Reviews of Effects (DARE; Other Reviews)
- EBSCO Host, including:
  - Academic Search Complete [1865 – 29 February 2008]
  - CINAHL (Cumulative Index to Nursing & Allied Health Literature) [1982 – 29 February 2008]
- ProQuest: ProQuest Medical Library [1986 – 29 February 2008]
- Scopus Abstracts [1996 – 29 February 2008]

### **2.3.2 Keywords for Searching**

The following keywords were identified and search string compiled, with the assistance of a qualified librarian, to ensure that all relevant studies were included in the initial search:

1. Trauma
2. Critically ill
3. Critical illness
4. Critical care

5. Intensive care
6. Intensive care unit\*
7. ICU
8. Acute phase response
9. Systemic inflammatory response syndrome
10. Compensatory anti-inflammatory response syndrome
11. SIRS
12. CARS
13. 1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8 OR 9 OR 10 OR 11 OR 12
14. Micronutrient\*
15. Vitamin\*
16. Trace element\*
17. Antioxidant\*
18. Nutrition\* support
19. Dietary supplement\*
20. Micronutrient supplement\*
21. Vitamin supplement\*
22. Trace element supplement\*
23. Antioxidant supplement\*
24. 14 OR 15 OR 16 OR 17 OR 18 OR 19 OR 20 OR 21 OR 22 OR 23
25. 13 AND 24

[\* All terms beginning with this root were included in the search (e.g. searching with the root *supplement* included terms such as supplementary, supplemented and supplementation).]

### **2.3.3 Study Selection Criteria**

Studies were selected for initial inclusion in this systematic review if they met the specified criteria (Table 2.1). Although the initial search (phase 1) used very broad search criteria and was not very specific (purposefully so), it did allow for the maximum number of potentially relevant studies to be identified. Once these were identified, stricter inclusion and exclusion criteria were applied (phase 2) to these studies for final inclusion (Table 2.2).

**Table 2.1: Initial search criteria (phase 1)**

<b>Study Population</b>	Adults Human Critically ill and related terms
<b>Intervention</b>	Micronutrient supplementation and related terms
<b>Study design</b>	Any
<b>Language</b>	All
<b>Search Period</b>	All

**Table 2.2: Inclusion and exclusion criteria for the final selection of studies (phase 2)**

Inclusion Criteria	Exclusion Criteria
Adults ( $\geq 14$ years)	Neonates and paediatric patients
Human studies	Animal studies
Critically ill patients	Not critically ill patients
Men and non-pregnant, non-lactating women	Pregnant / lactating women
Patients receiving micronutrient supplementation (enterally and/or parenterally)	Patients not receiving micronutrient supplementation OR Receiving micronutrients at the recommended doses as part of their total enteral or total parenteral nutrition therapy OR Receiving multiple nutrients (in addition to micronutrients)
All clinical studies reporting on 1 or more of the following: <ul style="list-style-type: none"> <li>• mortality</li> <li>• number of infectious complications</li> <li>• length of ICU stay (LICU)</li> <li>• length of hospital stay (LOS)</li> <li>• micronutrient status</li> <li>• morbidity</li> <li>• course of the APR as judged by <ul style="list-style-type: none"> <li>○ cytokine levels</li> <li>○ WBC and differential WBC count</li> <li>○ inflammatory markers (CRP)</li> </ul> </li> <li>• level of oxidative stress</li> </ul>	Outcomes not considered in the objectives
English language studies	Foreign language studies
Randomized controlled trials	All other study designs and (non)publication types, including: <ul style="list-style-type: none"> <li>• quasi-randomized controlled trials</li> <li>• review articles</li> <li>• unpublished studies / trials</li> <li>• abstracts / letters / editorials / comments</li> </ul>

Although only English language studies were included in this review, all potentially eligible studies reported in languages other than English were documented for future assessment (see Chapter 5: Conclusions and Recommendations).

## **2.4 METHODS OF THE REVIEW<sup>11</sup>**

After the completion of the initial literature search the citation lists (including titles and abstracts) were screened by the investigator. Studies that did not meet the initial inclusion criteria for the systematic review (Table 2.1) were excluded. For quality control purposes, two independent reviewers each randomly selected 10% of all the initial citations to ensure accuracy of the principle investigator. The two reviewers are qualified dietitians and were trained by the investigator in terms of systematic review processes, methodological quality assessment of trials and data extraction. Once the screening process was completed, the full text of the citations considered relevant for the review was retrieved by the investigator.

Upon retrieval of the relevant full articles, predetermined inclusion and exclusion criteria (Table 2.2) were applied to the full reference of all the studies to aid final selection. In addition to the investigator, the two independent reviewers undertook the final selection process, with disagreements resolved by discussion / consensus. The review process was blinded, with the investigator and two reviewers independently reviewing the articles to ensure that no relevant studies were excluded. Studies that initially appeared to be relevant but were subsequently excluded were listed in a table of excluded studies along with reasons for their exclusion. If two or more studies presented the same data from a single participant population, the data were included only once in the analysis.

### **2.4.1 Assessment of Quality of Evidence**

The methodological quality of all selected articles (phase 2) was assessed in duplicate (investigator and one reviewer), independently, using the components of a previously published system<sup>152</sup> (Table 2.3), also used in Cochrane reviews.<sup>151</sup> Disagreements were resolved within pairs by consensus.

**Table 2.3: Criteria used to assess methodological quality<sup>152</sup>**

Criteria	Score		
	0	1	2
<b>Randomization</b>	...	Not concealed or not sure	Concealed randomization
<b>Blinding</b>	Not blinded	...	Adjudicators blinded
<b>Analysis</b>	Other	...	Intention-to-treat
<b>Patient selection</b>	Selected patients or unable to tell	Consecutive eligible patients	...
<b>Comparability of groups at baseline</b>	No or not sure	Yes	...
<b>Extent of follow-up</b>	<100%	100%	...
<b>Treatment protocol</b>	Poorly described	Reproducibly described	...
<b>Co-interventions<sup>a</sup></b>	Not described	Described but not equal or not sure	Well described and all equal
<b>Outcomes</b>	Not described	Partially described	Objectively defined

The first 3 questions and the last 2 questions had a possible score of 0, 1, or 2. The middle 4 questions had a possible score of 0 or 1. The highest possible score was 14. Ellipses indicate data not applicable.

a: The extent to which non-trial interventions such as antibiotics, nutritional support, ventilation, oxygen, and transfusions were applied equally across groups.

Even in randomized trials, failure to prevent foreknowledge of treatment assignment can lead to overestimation of treatment effect.<sup>153</sup> The scoring system described above makes provision for this important aspect by scoring higher those studies that reported that their randomization scheme was concealed.

In addition, the Cochrane “Risk of bias” assessment tool<sup>11</sup> (Table 2.4) was completed for each of the included studies to further assess methodological quality and to enable data entry into the Review Manager 5.0 (RevMan 5)<sup>154</sup> programme.

**Table 2.4 Domains assessed in the Cochrane "Risk of bias" tool<sup>11</sup>**

<b>Domain</b>	<b>Description</b>	<b>Judgement</b>
<b>Sequence generation</b>	Describe the method used to generate the allocation sequence in sufficient detail to allow an assessment of whether it should produce comparable groups.	Was the allocation sequence adequately generated?
<b>Allocation concealment</b>	Describe the method used to conceal the allocation sequence in sufficient detail to determine whether intervention allocations could have been foreseen in advance of, or during, enrolment.	Was allocation adequately concealed?
<b>Blinding of participants, personnel and outcome assessors</b>	Describe all measures used, if any, to blind study participants and personnel from knowledge of which intervention a participant received. Provide any information relating to whether the intended blinding was effective.	Was knowledge of the allocated intervention adequately prevented during the study?
<b>Incomplete outcome data</b>	Describe the completeness of outcome data for each main outcome, including attrition and exclusions from the analysis. State whether attrition and exclusions were reported, the numbers in each intervention group (compared with total randomized participants), reasons for attrition/exclusions where reported, and any re-inclusions in analyses performed by the review authors.	Were incomplete outcome data adequately addressed?
<b>Selective outcome reporting</b>	State how the possibility of selective outcome reporting was examined by the review authors, and what was found.	Are reports of the study free of suggestion of selective outcome reporting?
<b>Other sources of bias</b>	State any important concerns about bias not addressed in the other domains in the tool.	Was the study apparently free of other problems that could put it at a high risk of bias?

For each of the included studies, each of these domains received a judgement of "Yes" (adequate - low risk of bias), "No" (inadequate - high risk of bias) or "Unclear" (unclear or unknown risk of bias).

### **2.4.2 Data Extraction**

Data extraction was carried out in duplicate (investigator and one reviewer), independently, and differences were resolved by consensus. Data extraction forms were designed to tabulate the characteristics of the included studies. Information

extracted included: study design and setting, characteristics of participants, details of interventions provided and details of outcomes evaluated. Where more than one publication relating to a particular trial existed, all publications were examined in order to obtain the most complete data set possible.

### **2.4.3 Prior Hypotheses Regarding Sources of Heterogeneity**

The planned sub-group analyses to explore possible sources of heterogeneity included:

- Studies of single micronutrient supplements compared to studies of combined/cocktail micronutrient supplements.
- Studies in which micronutrients were administered via the parenteral route compared to studies where micronutrients were administered via the enteral route (studies that used both routes of enteral and parenteral administration were excluded from the sub-group analyses of parenteral vs. enteral administration).
- Study quality based on concealment of allocation, intention-to-treat-analysis or level of blinding.

Given the awareness of existing data and a recent Cochrane Review (updated 2008)<sup>151</sup> regarding selenium supplementation in the critically ill adult, a sub-group analysis of selenium vs. non-selenium containing micronutrient supplements was not undertaken.

### **2.4.4 Data Synthesis / Analysis of Data**

The investigator was responsible for the preparation of the data for statistical analysis, using the RevMan 5<sup>154</sup> computer programme. The primary outcomes were mortality, number of infectious complications (pneumonia, line-related sepsis, and others as defined by the authors of the publications) and length of stay in ICU and hospital (in days). Data from all relevant studies were combined to estimate the common risk ratio (RR) and associated 95% confidence intervals (CI) for death and infectious complications (dichotomous data), and mean difference (MD) and associated 95% CI for LICU and LOS (continuous data). The random-effects model was used to estimate overall relative risk / mean difference and effect size due to the presence of study heterogeneity. Heterogeneity was expressed as the  $I^2$  statistic,<sup>155</sup> with a value of 0% indicating no heterogeneity and larger values showing increasing heterogeneity

where, for example, 50% suggested moderate heterogeneity. Funnel plots were used in exploratory data analyses to assess for the potential existence of publication bias for some of the main outcomes. All statistical methods utilized were confirmed by a statistician trained in meta-analyses. Post hoc it was noted that one small study<sup>167</sup> had a very short intervention duration (6 hours) and subsequently a sensitivity analysis was conducted removing this study from the aggregated results to evaluate the influence of this study on the overall conclusions. Differences at the level of  $p < 0.05$  was considered to be statistically significant. The secondary outcomes were sparse and variably recorded such that this data was not aggregated. Tables and graphs were used to describe these outcomes.

## **2.5 ETHICS AND LEGAL ASPECTS**

The research protocol for the study was approved by the Committee for Human Research, Faculty of Health Sciences, Stellenbosch University (99/035: 13/11/06; Appendix 6.1).

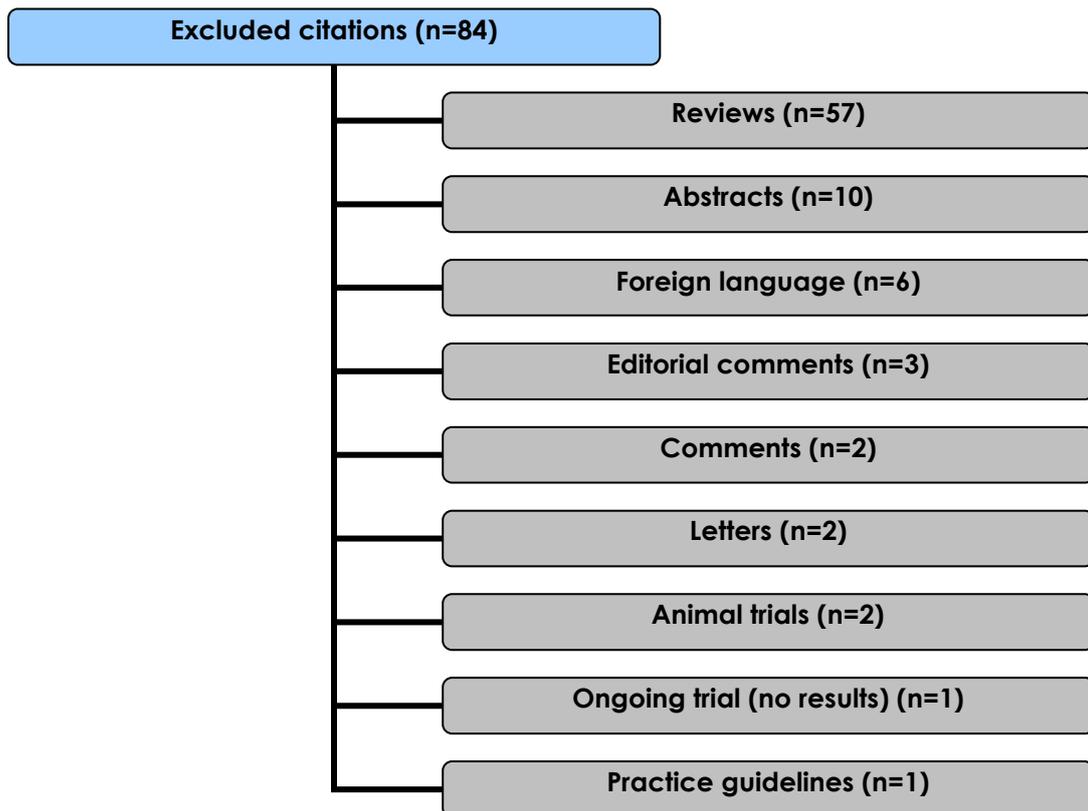
## **2.6 STUDY IDENTIFICATION AND SELECTION**

The initial literature search (phase 1) produced 1666 citations, of which 124 were selected by the investigator as potentially relevant citations (Table 2.5). These citations were selected according to the criteria specified for the review (Table 2.1). The two independent reviewers each randomly selected  $\pm 10\%$  ( $n=170$ ) of the citations for independent assessment with a subsequent 100% agreement with the investigator in terms of initial included/excluded studies. Additional citations were added ( $n=43$ ) as obtained from article reference lists and personal (private) files and all citations were corrected for duplicates ( $n=16$ ), providing a total of 151 citations for further assessment (phase 2).

**Table 2.5: Databases searched and citations identified (phase 1)**

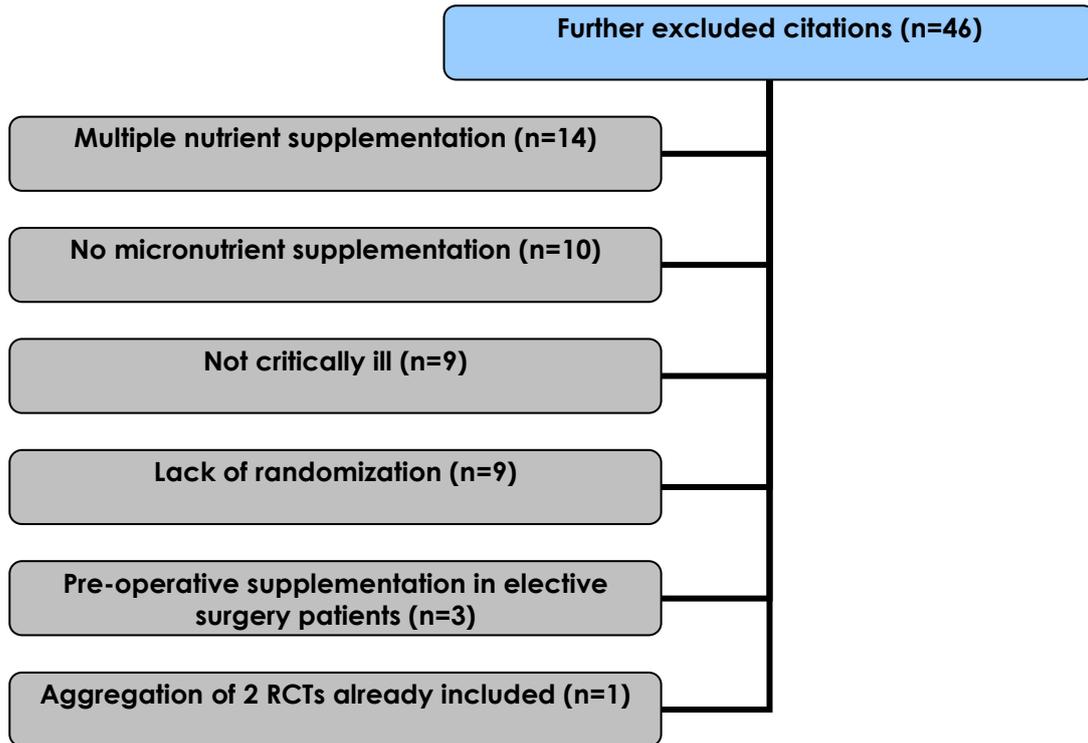
Electronic bibliographic database	Period searched	Total citations	Potentially useful citations
PUBMED/MEDLINE	1950 – 29/02/08	483	31
SCIENCE DIRECT	1823 – 29/02/08	467	25
ISI WEB OF SCIENCE	1987 – 29/02/08	388	18
COCHRANE CLINICAL TRIALS/CENTRAL	1800 – 29/02/08	125	36
COCHRANE REVIEWS/CDSR	1800 – 29/02/08	5	1
COCHRANE ABSTRACTS/DARE	1800 – 29/02/08	6	1
EBSCO HOST, including: ACADEMIC SEARCH COMPLETE CINAHL	1865 – 29/02/08 1982 – 29/02/08	113	7
PROQUEST MEDICAL LIBRARY	1986 – 29/02/08	60	4
SCOPUS ABSTRACTS	1996 – 29/02/08	19	1
<b>Sub-Total</b>		<b>1666</b>	<b>124</b>
Reference lists / Personal files			(+) 43
<b>Sub-Total</b>			<b>167</b>
Correction for duplicates			(-) 16
<b>TOTAL citations (phase 1)</b>			<b>151</b>

These 151 citations were independently reviewed by the investigator and the two independent reviewers using pre-designed study eligibility sheets (Appendix 6.2). Of the 151 citations identified 84 were immediately excluded by the investigator and the two independent reviewers. The main reasons for exclusion at this stage were review articles (n=57), abstracts (n=10) and foreign language trials (n=6) (Figure 2.1). The reference lists of these review articles were reviewed to ensure that all eligible studies were included. Although only English language studies were included in this review, all potentially eligible studies reported in languages other than English were documented for future assessment, if possible (see Chapter 5: Conclusions and Recommendations). Sixty-seven citations remained for in-depth assessment.



**Figure 2.1: Reasons for initial exclusion of citations**

Of the 67 publications identified for further assessment, 21 were included and 46 were excluded. The main reasons for study exclusion at this stage were multiple nutrient supplementation (n=14), no micronutrient supplementation (n=10), not critically ill (n=9) and lack of randomization (quasi-randomized studies) (n=9) (Figure 2.2). Detailed lists of the characteristics of excluded studies have been appended [Appendix 6.3 (reasons for study exclusion) and 6.4 (study identification numbers and bibliographic information)] for reference purposes. Similarly, study identification numbers, bibliographic information and sources of the 21 included publications have also been appended (Appendix 6.5).<sup>156-176</sup>



**Figure 2.2: Reasons for final exclusion of citations**

Of the 21 publications included, three trials had multiple full text publications [Angstwurm 2004 (S5)<sup>157</sup> and 1999 (S6)<sup>\*158</sup>; Berger 2001 (S14)<sup>\*159</sup> and 2001 (S26)<sup>162</sup>; Berger 2007 (S15)<sup>\*160</sup> and 2007 (S18)<sup>161</sup>; (\*: denotes the major publication for the study: these references will be used when referring to these trials in the rest of the text)]. As these studies presented the same data from a single participant population, the data were included only once in the analysis. Thus the data of 18 trials in total were included in this systematic review. Of the 18 trials identified via the search strategy, 15 (18 publications) provided outcomes for the primary objectives of this study, whilst all 18 trials (21 publications) provided outcomes for the secondary objectives of this study (Figure 2.3).

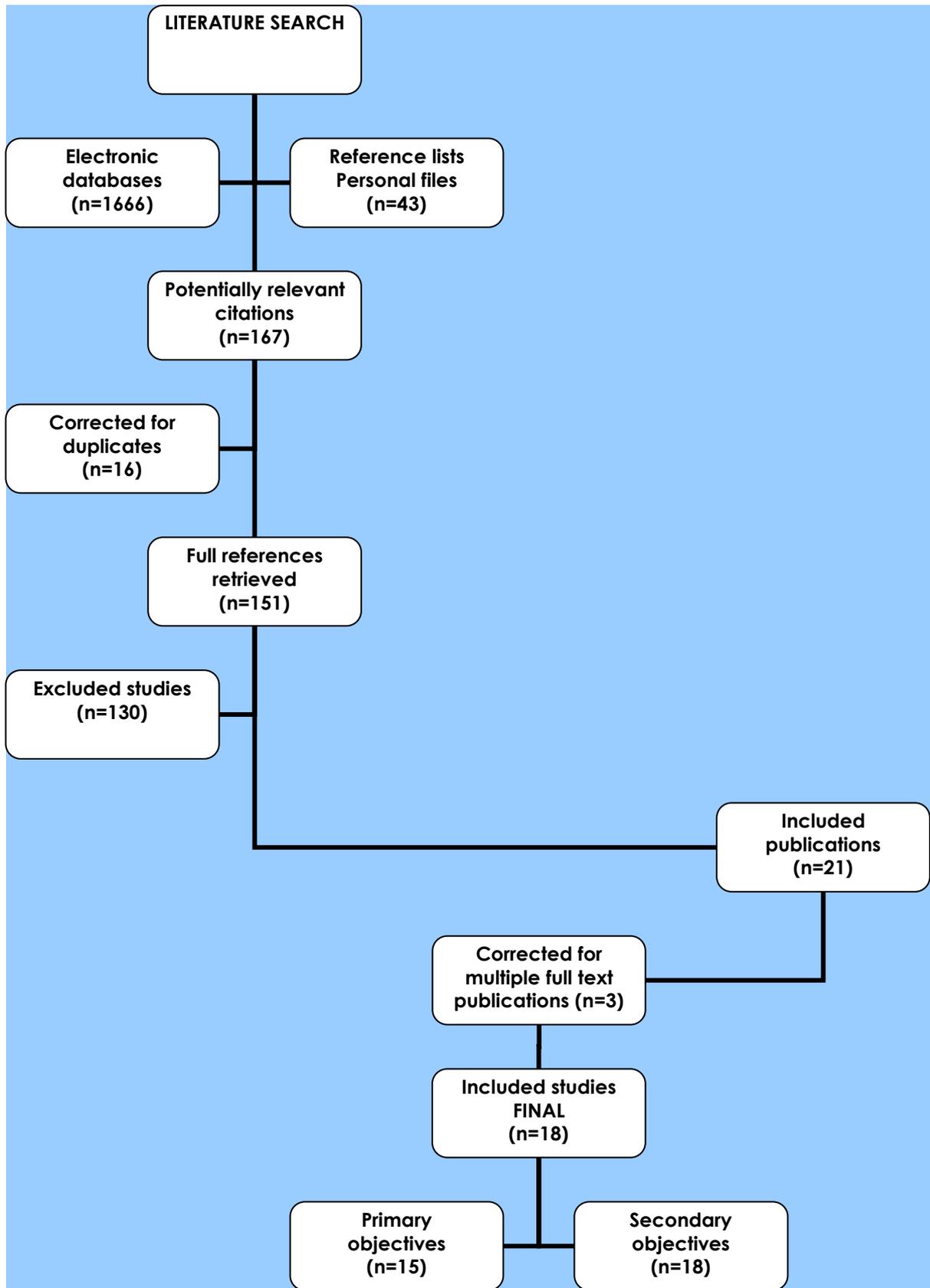


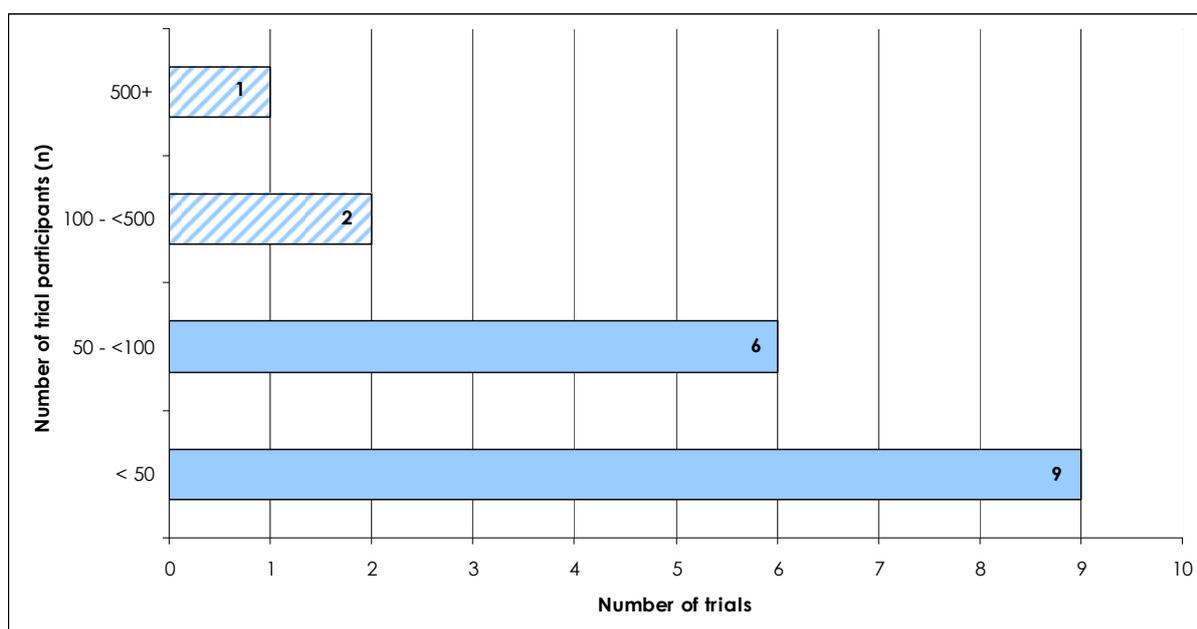
Figure 2.3: Diagrammatic representation of the process followed in the selection of studies

## **CHAPTER 3: RESULTS**

### 3.1 DESCRIPTION OF STUDIES

#### 3.1.1 General Description

The included trials were all published between 1996 and 2007 except for one trial that was published in 1991.<sup>168</sup> Four trials were multi-centre trials,<sup>156,165,166,175</sup> with the remaining 14 being single centre trials.<sup>158-160,163,164,167-174,176</sup> The 18 trials included in the review involved a total of 1849 participants. The majority of trials included less than 50 patients (n=9), with only three trials recruiting more than 100 patients (Figure 3.1).<sup>156,165,170</sup> The details of the included trials are provided in the table “Characteristics of included trials” (Appendix 6.6).



**Figure 3.1: The number of trials by number of included participants**

Thirteen trials recruited more male than female participants.<sup>156,158-160,164-168,170,172,173,176</sup> One trial recruited only men,<sup>171</sup> two did not report the gender of the participants<sup>163,174</sup> and two studies had more women than men.<sup>169,175</sup> Where reported, the mean age of the participants was more than 30 years, except for one study that recruited younger patients.<sup>168</sup> Trials of micronutrient supplementation recruited participants with the following conditions: trauma (medical and/or surgical ICU patients),<sup>159,164,168,170-172,174</sup> sepsis, septic shock or SIRS,<sup>156,158,166,167,169</sup> burns,<sup>160,163,173</sup> severe acute pancreatitis,<sup>175</sup> head injury<sup>176</sup> and coronary critical care patients.<sup>165</sup> Other study characteristics are discussed under the relevant results sections.

### 3.1.2 Methodological Quality

All included trials were RCTs; no quasi-randomized clinical trials were included. The full details of the methodological quality of studies are provided in Appendix 6.7. As previously stated two assessment tools were utilized to assess study quality. The scoring system introduced by Heyland et al.<sup>152</sup> (refer to Table 2.3) indicated scores ranging from 4 to 13 out of a total of 14 (Table 3.1). Half of the studies (n=9) obtained a score of 10 or more (Table 3.1). The average score of the 18 trials was 9.8 [standard deviation (SD) 2.3].

**Table 3.1: Quality assessment of studies based on a scoring system<sup>152</sup>**

Study, Year and Identification numbers (S)	Score (/14)
Angstwurm 2007 (S3)	9
Angstwurm 2004 & 1999* (S5 & S6*)	11
Berger 2001 & 2001 (S14* & S26)	12
Berger 2007 & 2007 (S15* & S18)	9
Berger 1998 (S33)	12
Cheng 2006 (S52)	10
Crimi 2004 (S54)	13
Forceville 2007 (S65)	10
Galley 1997 (S68)	8
Maderazo 1991 (S92)	9
Mishra 2007 (S103)	12
Nathens 2002 (S107)	7
Porter 1999 (S115)	9
Preiser 2000 (S118)	8
Rock 1997 (S124)	9
Rümelin 2005 (S128)	4
Siriwardena 2007 (S135)	13
Young 1996 (S146)	12
<b>Mean (SD)</b>	<b>9.8 (2.3)</b>

\*: Indicates the major publication for the study

In addition the Cochrane “Risk of bias” assessment tool<sup>11</sup> was completed for each of the included studies (Appendix 6.7) to further assess methodological quality and to enable data entry into the RevMan 5<sup>154</sup> programme. This tool was primarily important for the 15 trials included for the primary objectives and the meta-analysis.<sup>156,158-</sup>

<sup>160,163,165-172,175,176</sup> The quality assessment of the trial methodology reported for each of these 15 trials (Figure 3.2) indicates that concealment of allocation was confirmed in only seven of the 15 trials: Cochrane allocation concealment score A.<sup>159,163,165,166,168,171,175</sup> The other trials did not clearly report the method of concealment of allocation: Cochrane allocation concealment score B. Although not always explicitly stated, intention-to-treat analysis was undertaken in the majority (n=12) of trials, with only three trials undertaking per-protocol analyses.<sup>156,170,172</sup> Ten trials reported to be blinded or double blinded.<sup>156,159,163,165,166,168,169,172,175,176</sup> Only two trials clearly described recruiting consecutive eligible patients.<sup>158,165</sup>

	Adequate sequence generation?	Allocation concealment?	Blinding?	Incomplete outcome data addressed?	Free of selective reporting?
Angstwurm 1999	?	?	-	+	+
Angstwurm 2007	+	?	+	-	+
Berger 1998	?	+	+	+	+
Berger 2001	?	+	+	+	+
Berger 2007	?	?	?	+	-
Crimi 2004	+	+	+	+	-
Forceville 2007	?	+	+	+	+
Galley 1997	?	?	-	+	+
Maderazo 1991	?	+	+	+	+
Mishra 2007	?	?	+	+	+
Nathens 2002	+	?	-	-	+
Porter 1999	?	+	-	+	+
Preiser 2000	+	?	+	-	+
Siriwardena 2007	+	+	+	+	+
Young 1996	+	?	+	+	+

**Key**

?	+	-
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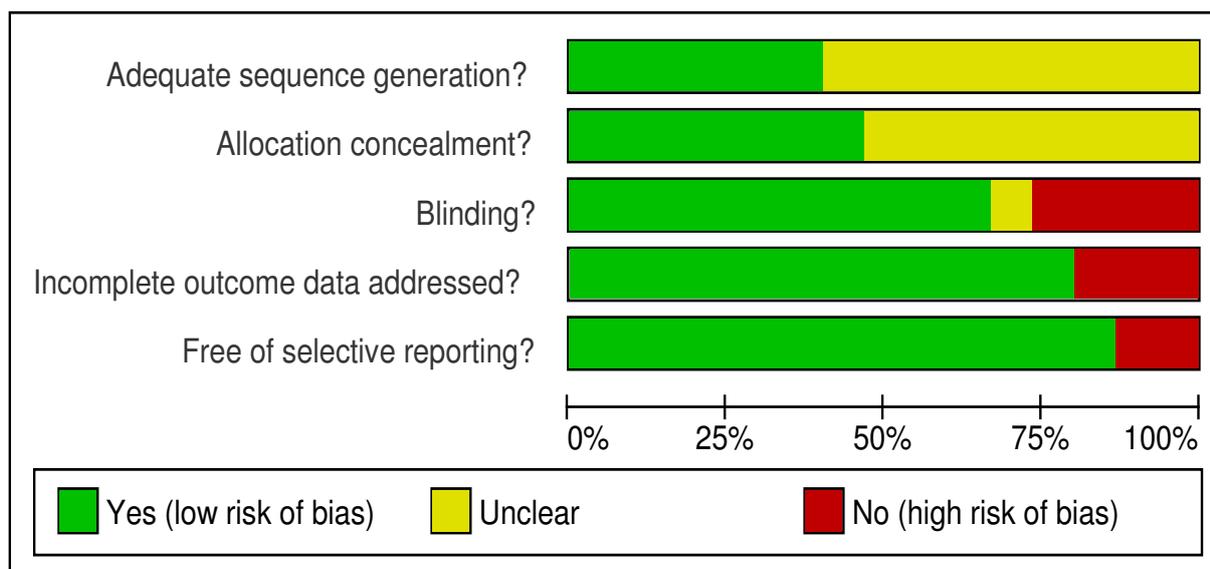
Unclear (uncertain risk of bias)

Yes (low risk of bias)

No (high risk of bias)

**Figure 3.2: Methodological quality summary: judgements about each methodological quality item for each included study**

The investigator and reviewer judgements about each methodological quality assessment factor across all included studies for the primary objectives (Figure 3.3) indicate uncertainty (unclear risk of bias) in especially the sequence generation and allocation concealment categories.



**Figure 3.3: Methodological quality graph: judgements about each methodological quality item presented as percentages across all included studies**

### 3.2 PRIMARY OBJECTIVES

Fifteen studies out of the 18 included studies provided outcomes related to the primary objectives of the review (Table 3.2).<sup>156,158-160,163,165-172,175,176</sup> All results in this section were obtained from these 15 trials, which included 1714 participants (randomized) of whom 1514 were analyzed in the relevant studies (the latter due to per-protocol analyses undertaken in three trials).<sup>156,170,172</sup> Where available, results have been presented using denominators based on the number of participants at randomization.

**Table 3.2: Results of randomized trials evaluating micronutrient strategies in critically ill patients (primary objectives)**

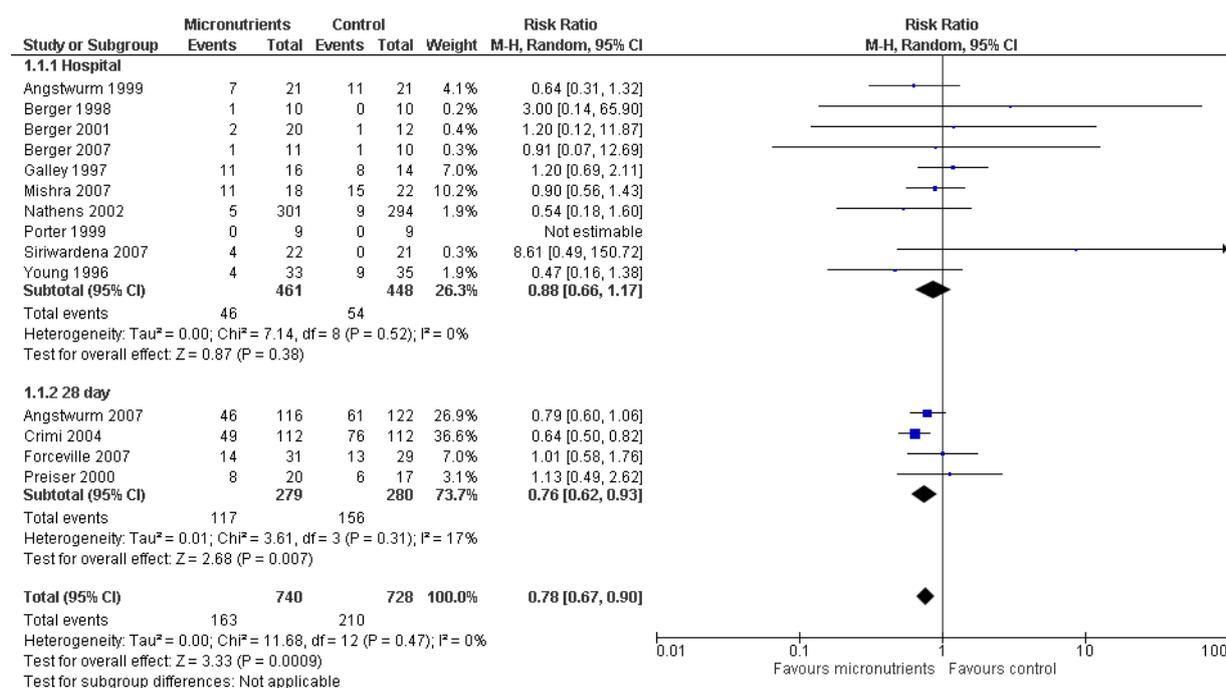
Study, Year & Study ID	Mortality <sup>a</sup>			Infections <sup>b</sup>			Length of stay (days)		
	Experimental	Control	p	Experimental	Control	p	Experimental	Control	p
Angstwurm 2007 (S3)	28 day: 46/116 (39.7%)	28 day: 61/122 (50.0%)	0.109 (OR 0.66; CI 0.39-1.1)	10/116 (8.6%)	10/122 (8.2%)	NS	ICU: 15.1±10 <sup>c</sup>	ICU: 12.7±9 <sup>c</sup>	NS
Angstwurm 2004 & 1999* (S5 & S6*)	7/21 (33%)	11/21 (52%)	0.135	NA	NA	-	ICU Rx: 14 (3-75) <sup>d</sup>	ICU Rx: 11 (2-67) <sup>d</sup>	NA, seems NS for both
Berger 2001 & 2001 (S14* & S26)	Se <sup>only</sup> : 2/9 (22%) Se <sup>plus</sup> : 0/11 (0%)	1/12 (8%)	NS	Se <sup>only</sup> : 5/9 (56%) Se <sup>plus</sup> : 3/11 (27%)	5/12 (42%)	NS	Se <sup>only</sup> : ICU: 8.0±4.0 <sup>c</sup> Hospital: 82±78 <sup>c</sup> Se <sup>plus</sup> : ICU: 5.8±4.4 <sup>c</sup> Hospital: 60±48 <sup>c</sup> Combined: ICU: 6.8±4.3 <sup>c</sup> Hospital: 69.9±62.5 <sup>c</sup>	ICU: 8.6±8.1 <sup>c</sup> Hospital: 64±39 <sup>c</sup>	NS
Berger 2007 & 2007 (S15* & S18)	1/11 (9.1%)	1/10 (10.0%)	NS	2.1±1.0 (2) <sup>e</sup> per patient 23 episodes in total: 30 d. (1-4) <sup>f</sup>	3.6±1.3 (4) <sup>e</sup> per patient 36 episodes in total: 30 d. (1-5) <sup>f</sup>	0.01  0.015	ICU: 35±27 <sup>c</sup> 23 (6-91) <sup>d</sup> Hospital: no details provided	ICU: 47±37 <sup>c</sup> 38 (16-145) <sup>d</sup> Hospital: no details provided	NS  NS
Berger 1998 (S33)	1/10 (10%)	0/10 (0%)	NA	1.9±0.9 (1-4) <sup>g</sup> per patient	3.1±1.1 (2-5) <sup>g</sup> per patient	0.013	ICU: 30±12 (14-46) <sup>g</sup> Hospital: 54±27 (26-94) <sup>g</sup>	ICU: 39±13 (18-58) <sup>g</sup> Hospital: 66±31 (24-129) <sup>g</sup>	NS
Crimi 2004 (S54)	28 day: 49/112 (43.7%)	28 day: 76/112 (67.8%)	<0.05	No details provided	No details provided	NS	Hospital: 26.5 <sup>h</sup>	Hospital: 27.5 <sup>h</sup>	NS
Forceville 2007 (S65)	28 day: 14/31 (45%) 6 m: 21/31 (68%) 1 yr: 22/31 (71%)	28 day: 13/29 (45%) 6 m: 17/29 (59%) 1 yr: 19/29 (66%)	0.59  0.32  0.43	17/31 (55%)	13/29 (45%)	0.438	ICU: 21 (7-40) <sup>i</sup> Hospital: 25 (7-68) <sup>i</sup>	ICU: 18 (10-31) <sup>i</sup> Hospital: 33 (11-51) <sup>i</sup>	0.836  0.704

Study, Year & Study ID	Mortality <sup>a</sup>			Infections <sup>b</sup>			Length of stay (days)		
	Experimental	Control	p	Experimental	Control	p	Experimental	Control	p
Galley 1997 (S68)	ICU: 11/16 (68.8%)	ICU: 8/14 (57.1%)	NS	NA	NA	-	NA	NA	-
Maderazo 1991 (S92)	NA	NA	-	13/28 (46%)	5/18 (28%)	NA	NA	NA	-
Mishra 2007 (S103)	Hospital: 11/18 (61%) 28 day: 8/18 (44%)	Hospital: 15/22 (68%) 28 day: 11/22 (50%)	0.89 0.97	1.5 (1.9) <sup>j</sup> per patient	1.8 (1.6) <sup>j</sup> per patient	0.6	ICU: 21.3 (16.2) <sup>j</sup>	ICU: 20.8 (21.8) <sup>j</sup>	0.94
Nathens 2002 (S107)	ICU: 3/301 (1.0%) Hospital: 5/301 (1.7%), 28 day: 4/301 (1.3%)	ICU: 9/294 (3.1%) Hospital: 9/294 (3.1%), 28 day: 7/294 (2.4%)	NA	36/301 (11.9%)	44/294 (15.0%)	NA	ICU: 5.3 <sup>h</sup> Hospital: 14.6 <sup>h</sup>	ICU: 6.4 <sup>h</sup> Hospital: 15.1 <sup>h</sup>	NA
Porter 1999 (S115)	0/9 (0%)	0/9 (0%)	NA	5/9 (56%) 8 (nr: infections)	8/9 (89%) 18 (nr: infections)	NA	ICU: 22.0±25.2 <sup>c</sup> Hospital: 31.3±23.4 <sup>c</sup>	ICU: 35.8±24.9 <sup>c</sup> Hospital: 49.0±30.0 <sup>c</sup>	<0.05
Preiser 2000 (S118)	ICU: 3/20 (15%) 28 day: 8/20 (40%)	ICU: 3/17 (18%) 28 day: 6/17 (35%)	NS	3/20 (15%)	1/17 (6%)	NS	ICU: 5 (3-26) <sup>d</sup>	ICU: 5 (3-18) <sup>d</sup>	NS
Siriwardena 2007 (S135)	4/22 (18.2%)	0/21 (0%)	0.108	NA	NA	-	ICU: 4.0 (10.3) <sup>j</sup> Hospital: 20.4 (24.4) <sup>j</sup>	ICU: 0.0 (0.0) <sup>j</sup> Hospital: 14.3 (15.7) <sup>j</sup>	0.084 0.34
Young 1996 (S146)	4/33 (12%)	9/35 (26%)	0.09	NA	NA	-	NA	NA	-

\* Indicates the major publication for the study; <sup>a</sup> Mortality: Presumed hospital mortality unless otherwise specified; <sup>b</sup> Refers to the number of patients with infections unless specified; <sup>c</sup> X ± SD: Mean ± Standard Deviation (SD); <sup>d</sup> Median (range in parentheses); <sup>e</sup> Mean ± SD (Median in parentheses); <sup>f</sup> Range in parentheses; <sup>g</sup> Mean ± SD (Range in parentheses); <sup>h</sup> Mean; <sup>i</sup> Median (interquartile range in parentheses); <sup>j</sup> Mean(SD); NA: Not available; NS: Not significant; Porter 1999: Standard error converted to standard deviation as follows: SE = s (SD) / square root of n (number of items in sample); Berger 2001: LICU and LOS combined determined using the formula: S = Square root of:  $\frac{\sum \chi^2 - (\sum \chi)^2 / n}{n-1}$

### 3.2.1 Mortality

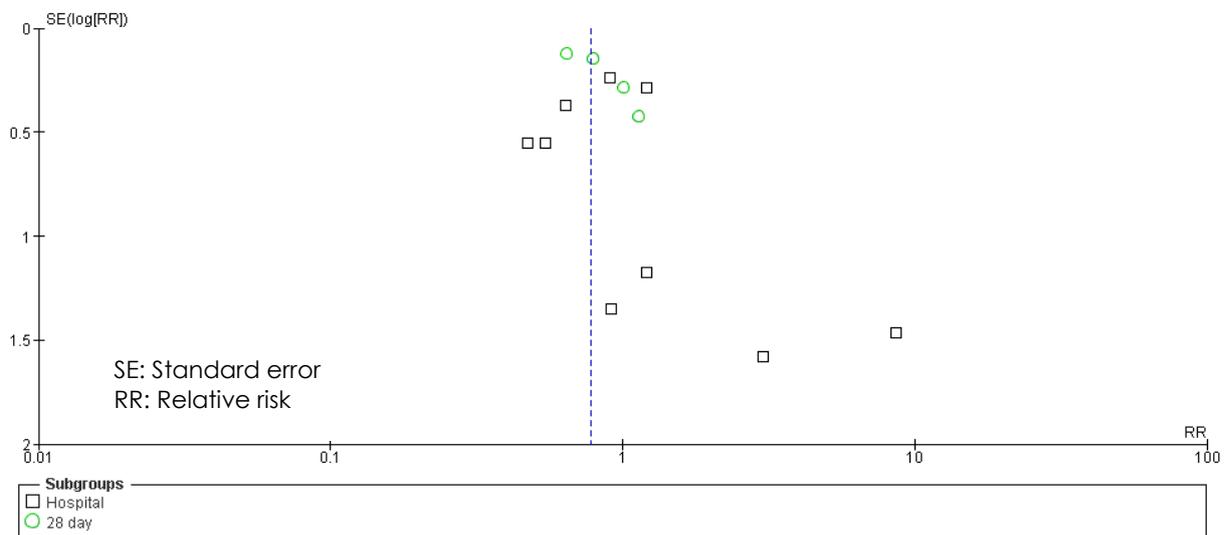
Fourteen out of the 15 trials provided mortality data (dichotomous data).<sup>156,158-160,163,165-167,169,170-172,175,176</sup> These trials reported at least hospital<sup>158,159,160,163,167,171,175,176</sup> or 28 day<sup>156,165,166,172</sup> mortality, whilst two trials<sup>169,170</sup> provided both hospital *and* 28 day mortality. For overall effect on mortality, hospital mortality was included where available (10 trials), whilst 28 day mortality was included for the remaining four trials.<sup>156,165,166,172</sup> Thus for the two trials that reported both hospital and 28 day mortality, only mortality assessed after the longest duration of time (i.e. hospital mortality) was included in the analysis for overall mortality. When the results of all 14 RCTs were aggregated (n=1468), overall micronutrients were associated with a significant reduction in mortality [Risk Ratio (RR) 0.78, 95% Confidence Interval (CI) 0.67-0.90, p=0.0009; see Figure 3.4]. No evidence of statistical heterogeneity was present (I<sup>2</sup>=0%).



**Figure 3.4: Effect of micronutrient supplementation on overall mortality in critically ill patients**

Visual inspection of a funnel plot for studies using micronutrients and its effect on mortality (Figure 3.5) indicates a skewed (asymmetrical) funnel, necessitating caution when directly interpreting the overall mortality results. Publication or other sources of bias (e.g. with respect to study size and poor methodological design of small studies)

could, to some extent, account for some of the apparent benefit observed with respect to overall mortality.

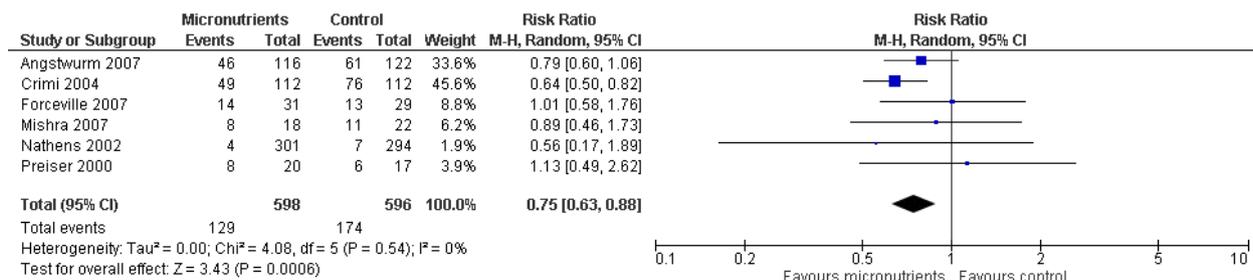


**Figure 3.5: Funnel plot for studies using micronutrients (overall mortality)**

In a sensitivity analysis one study with a very short intervention duration (6 hours)<sup>167</sup> was excluded, and the overall estimate of treatment effect did not change appreciably, with micronutrient supplementation still associated with a significant reduction in overall mortality [adjusted RR without (Galley 1997)<sup>167</sup> 0.75, 95% CI 0.64-0.88,  $I^2=0\%$ ,  $p=0.0003$ ].

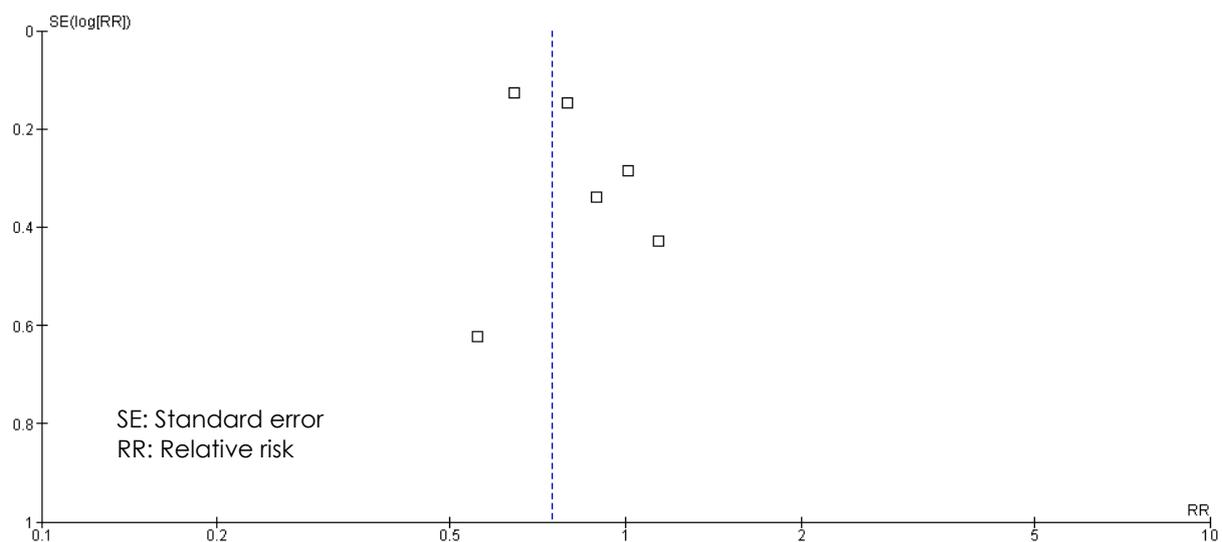
Hospital mortality on its own showed a relative risk of 0.88 (95% CI 0.66-1.17,  $I^2=0\%$ ,  $p=0.38$ ; Figure 3.4), indicating an insignificant reduction. Again, in a sensitivity analysis Galley 1997<sup>167</sup> was removed from the results, and the overall estimate of treatment effect did not change appreciably, with micronutrient supplementation still having no significant effect on hospital mortality [adjusted RR without (Galley 1997)<sup>167</sup> 0.78, 95% CI 0.56-1.10,  $I^2=0\%$ ,  $p=0.16$ ].

Six trials in total<sup>156,165,166,169,170,172</sup> reported on 28 day mortality and when the results of these trials were aggregated ( $n=1194$ ), micronutrient supplementation was associated with a significant reduction in 28 day mortality (RR 0.75, 95% CI 0.63-0.88,  $p=0.0006$ ; Figure 3.6). Again no evidence of statistical heterogeneity was present ( $I^2=0\%$ ).



**Figure 3.6:** Effect of micronutrient supplementation on 28 day mortality in critically ill patients

Visual inspection of a funnel plot for studies using micronutrients and its effect on 28 day mortality (Figure 3.7) indicates a more symmetrical (inverted) funnel, demonstrating that there is little or no indication that a publication or other bias accounts for the apparent benefit observed.



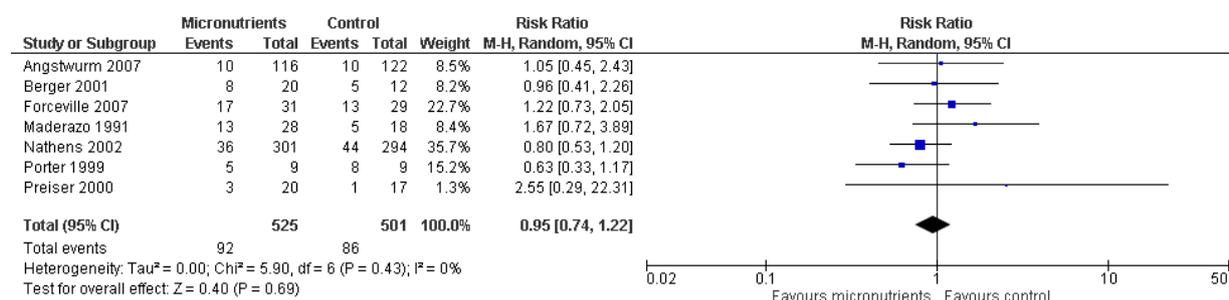
**Figure 3.7:** Funnel plot for studies using micronutrients (28 day mortality)

Only two trials<sup>170,172</sup> reported on ICU mortality, and when the results of these trials were aggregated (n=632), it indicated a relative risk of 0.50 (95% CI 0.19-1.31, I<sup>2</sup>=0%, p=0.16), an insignificant finding.

### 3.2.2 Infectious Complications

Although 11 out of the 15 trials provided infectious complications data (Table 3.2), only seven trials (n=1026)<sup>156,159,166,168,170-172</sup> reported infectious complications as number of patients with infections (dichotomous data). Pooling these seven trials indicated

that micronutrient supplements showed no significant effect on infectious complications (RR 0.95, 95% CI 0.74-1.22,  $I^2=0\%$ ,  $p=0.69$ ; Figure 3.8).

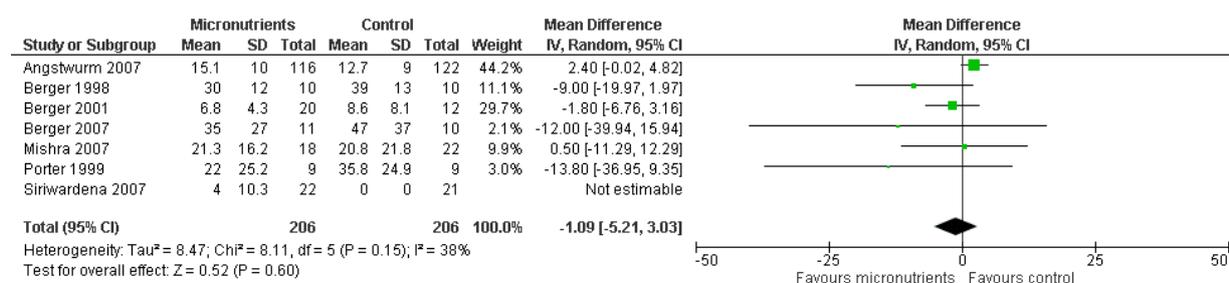


**Figure 3.8: Effect of micronutrient supplementation on number of infected critically ill patients**

Of the four remaining trials that could not be included for analysis, three provided data in the form of number of infections per patient,<sup>160,163,169</sup> with Berger 2007 and 1998 reporting significant differences between groups ( $p=0.01$  and  $p=0.013$  respectively) and Mishra 2007 reporting no significant difference between groups ( $p=0.6$ ). The one remaining trial<sup>165</sup> indicated no statistically significant difference between the micronutrient supplemented and control group, but provided no data to consider for inclusion in the meta-analysis.

### 3.2.3 Length of Intensive Care Unit Stay (LICU)

Although 11 out of the 15 trials provided data on LICU (Table 3.2), only seven trials<sup>156,159,160,163,169,171,175</sup> reported LICU as mean  $\pm$  standard deviations (SD) (continuous data). When the results of these seven trials were aggregated ( $n=412$ ), micronutrient supplementation was not associated with a significant difference in LICU (MD -1.09, 95% CI -5.21-3.03,  $I^2=38\%$ ,  $p=0.60$ ; Figure 3.9).

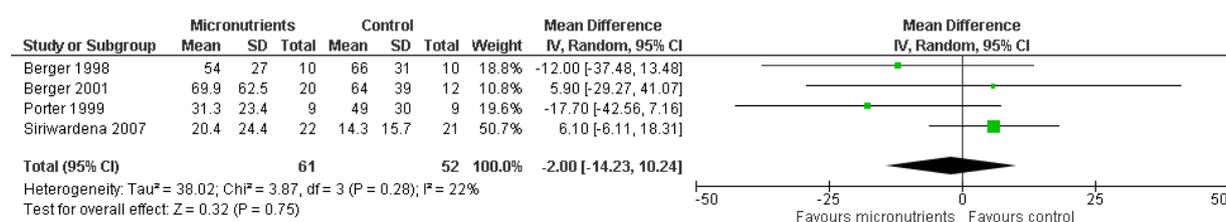


**Figure 3.9: Effect of micronutrient supplementation on length of intensive care unit stay**

Of the four remaining trials that could not be included for analysis, two provided data in the form of medians (with ranges in parentheses),<sup>158,172</sup> one provided medians (with interquartile ranges in parentheses)<sup>166</sup> and one provided means only.<sup>170</sup> In Angstwurm 1999<sup>158</sup> the supplemented group stayed for a median of 14 days (range 2-75 days) while the control group stayed a median of 11 days (range 2-67 days) (no p value reported). In Preiser 2000<sup>172</sup> the supplemented group stayed for a median of 5 days (range 3-26 days) while the control group also stayed for a median of 5 days (with a range of 3-18 days) (this was reported as not significantly different). Forceville 2007<sup>166</sup> reported LICU as 21 (7-40) and 18 (10-31) respectively for the supplemented and control groups (as medians with interquartile ranges) (p=0.836). Nathens 2002<sup>170</sup> reported LICU as 5.3 and 6.4 (means) respectively for the supplemented and control groups (no p value was reported).

### 3.2.4 Length of Hospital Stay (LOS)

Eight out of the 15 trials provided data on LOS (Table 3.2), with only four trials<sup>159,163,171,175</sup> reporting LOS as mean  $\pm$  standard deviations (SD) (continuous data) that could be extracted and aggregated for analysis. When the results of these four trials were pooled (n=113), micronutrient supplementation was not associated with a significant difference in LOS (MD -2.00, 95% CI -14.23-10.24, I<sup>2</sup>=22%, p=0.75; Figure 3.10).



**Figure 3.10: Effect of micronutrient supplementation on length of hospital stay**

Of the four remaining trials that could not be included for analysis, two provided means only,<sup>165,170</sup> one provided medians (with interquartile ranges in parentheses)<sup>166</sup> and one indicated no statistically significant difference between the micronutrient supplemented and control group, but provided no data to consider for inclusion in the meta-analysis.<sup>160</sup> Crimi 2004<sup>165</sup> reported LOS as 26.5 and 27.5 (means) respectively for the supplemented and control groups (this was reported as not significantly different). Similarly Nathens 2002<sup>170</sup> reported LOS as 14.6 and 15.1 (means)

respectively for the supplemented and control groups (but in this case no p value was reported). Forceville 2007<sup>166</sup> reported LOS as 25 (7-68) and 33 (11-51) respectively for the supplemented and control groups (as medians with interquartile ranges) ( $p=0.704$ ).

### **3.2.5 Subgroup Analysis**

In the 15 trials of micronutrient intervention there was great variation in the type of micronutrients studied, the route of administration, the dosage and the effect of other combination nutrients, thereby making it difficult to attribute any effects to a single nutrient or combination of micronutrients. Several pre-planned subgroup analyses (see section 2.4.3) were carried out in an attempt to determine which micronutrient strategies were more likely to affect clinical outcomes.

The planned sub-group analyses to explore possible sources of heterogeneity included:

- Single nutrients versus combined/cocktail micronutrient supplements
- Parenteral/intravenous versus enteral route
- Study quality based on concealment of allocation, intention-to-treat-analysis or level of blinding

#### **3.2.5.1 Single nutrients versus combined/cocktail micronutrient supplements**

As previously stated, given the awareness of existing data and a recent Cochrane Review (updated 2008)<sup>151</sup> regarding selenium supplementation in the critically ill adult, a sub-group analysis of selenium vs. non-selenium containing micronutrient supplements was not undertaken, although the outcome of this systematic review will be taken up in the discussion section.

For the various subgroup analyses regarding single nutrients versus combined/cocktail micronutrient supplements, the Berger 2001 trial<sup>159</sup> was divided into two (Berger 2001a and Berger 2001b) as this trial included 2 treatment groups: i.e. a selenium only supplemented group (Berger 2001a; single nutrient) and a selenium plus (copper, zinc,  $\alpha$ -tocopherol) supplemented group (Berger 2001b; combined/cocktail supplement). Six of the 15 trials used single micronutrients (including the single nutrient intervention group of Berger 2001), whilst ten trials

provided combined/cocktail micronutrient supplements (including the combined micronutrient intervention group of Berger 2001) (Table 3.3). Five of the six trials using single micronutrients, provided treatment in the form of selenium (sodium selenite), with the remaining trial providing zinc in head injured patients.

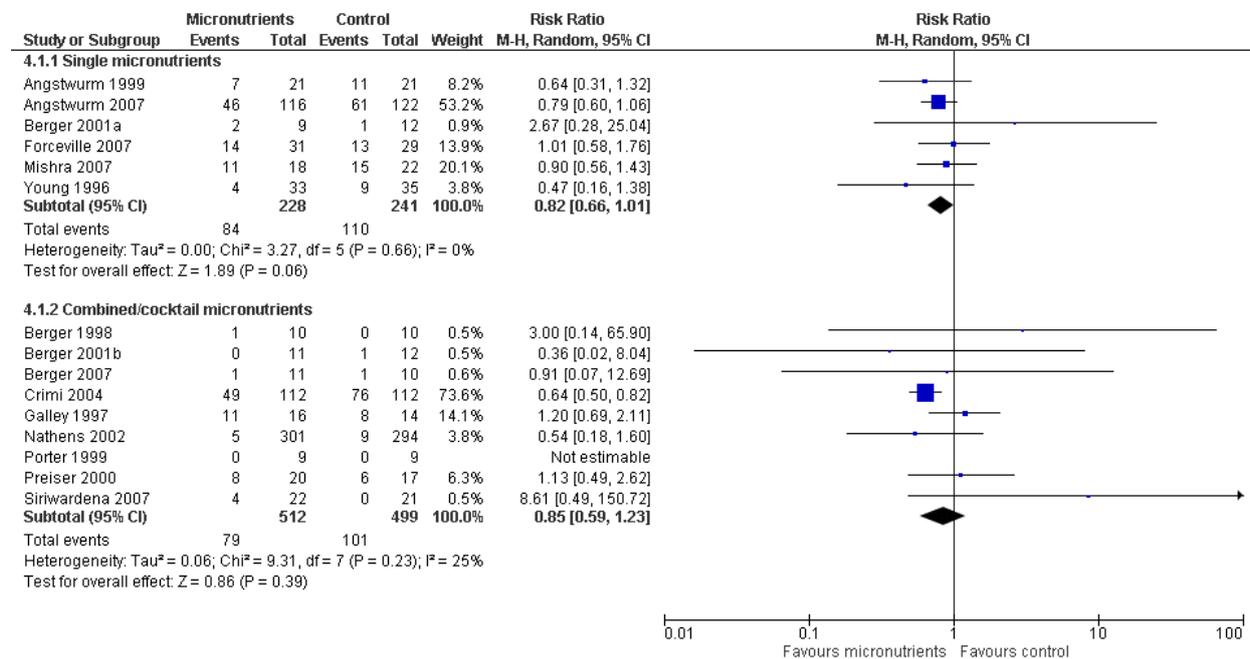
**Table 3.3: Trial summary: single versus combined/cocktail micronutrient supplements**

Single nutrients		Combined micronutrients	
Study	Micronutrient	Study	Micronutrients
Angstwurm 2007 (S3)	Selenium	Berger 2001b (S14)	Selenium Copper Zinc $\alpha$ -tocopherol
Angstwurm 1999 (S6)	Selenium	Berger 2007 (S15)	Selenium Copper Zinc
Berger 2001a (S14)	Selenium	Berger 1998 (S33)	Selenium Copper Zinc
Forceville 2007 (S65)	Selenium	Crimi 2004 (S54)	Ascorbic acid $\alpha$ -tocopherol
Mishra 2007 (S103)	Selenium	Galley 1997 (S68)	Ascorbic acid $\alpha$ -tocopherol
Young 1996 (S146)	Zinc	Maderazo 1991 (S92)	Ascorbic acid $\alpha$ -tocopherol
		Nathens 2002 (S107)	Ascorbic acid $\alpha$ -tocopherol
		Porter 1999 (S115)	Selenium Ascorbic acid $\alpha$ -tocopherol
		Preiser 2000 (S118)	Vitamin A Ascorbic acid $\alpha$ -tocopherol
		Siriwardena 2007 (S135)	Selenium Ascorbic acid

### 3.2.5.1.1 Effect of single nutrients versus combined micronutrient supplements on mortality

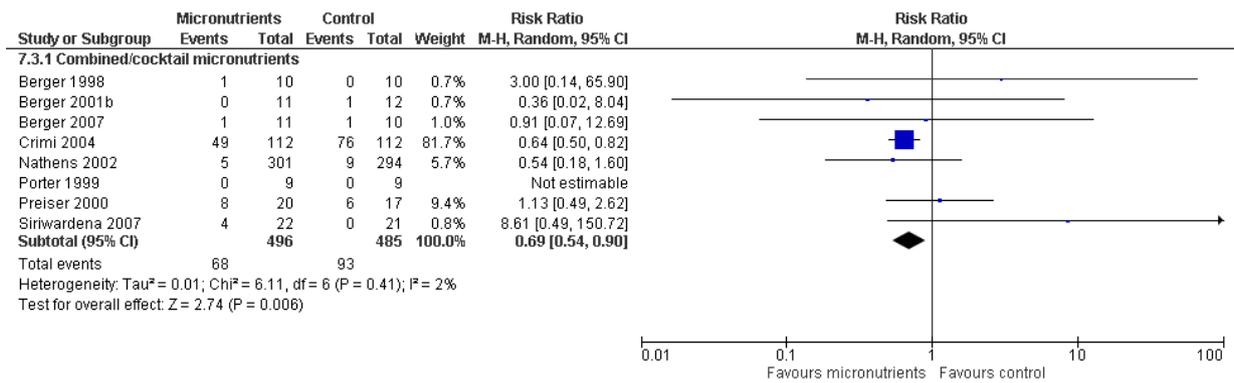
All six of the single nutrient studies<sup>156,158,159,166,169,176</sup> and nine of the combined micronutrient studies<sup>159,160,163,165,167,170-172,175</sup> reported mortality data. When the results of the six studies using single micronutrients were aggregated (n=469), statistical

significance in terms of effect on mortality was borderline (RR 0.82, 95% CI 0.66-1.01,  $I^2=0\%$ ,  $p=0.06$ ; Figure 3.11).



**Figure 3.11: Effect of single nutrients versus combined micronutrient supplements on mortality**

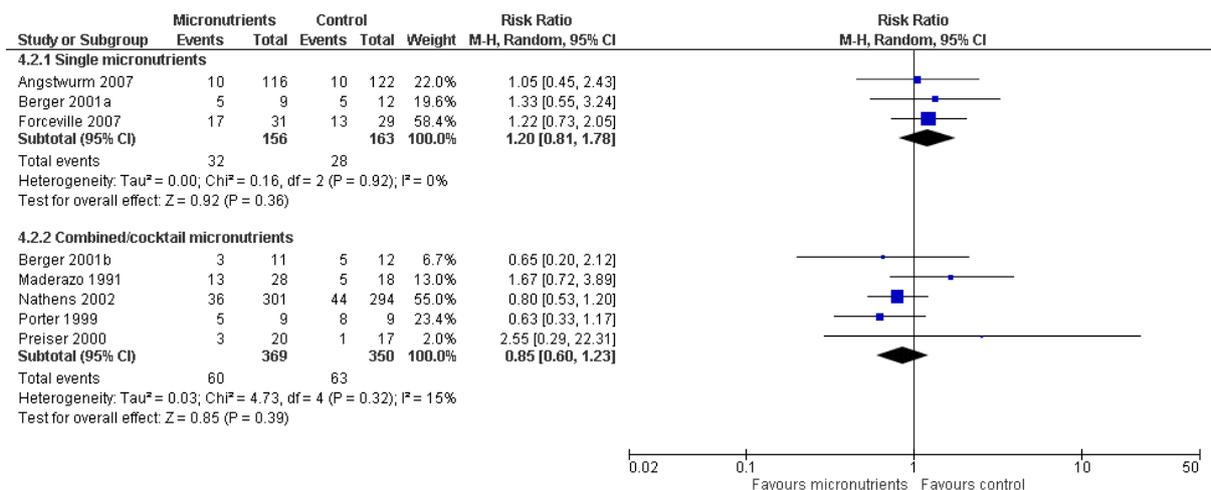
When the results of the nine studies using combined micronutrients were aggregated ( $n=1011$ ), combined micronutrient supplementation was not associated with a significant difference in mortality (RR 0.85, 95% CI 0.59-1.23,  $I^2=25\%$ ,  $p=0.39$ ; Figure 3.11). In a sensitivity analysis one study with a very short intervention duration (6 hours)<sup>167</sup> was excluded, and the overall estimate of treatment effect changed appreciably, with combined micronutrient supplementation now associated with a significant reduction in overall mortality [adjusted RR without (Galley 1997)<sup>167</sup> 0.69, 95% CI 0.54-0.90,  $I^2=2\%$ ,  $p=0.006$ ; Figure 3.12].



**Figure 3.12: Sensitivity analysis: Effect of combined micronutrient supplements on mortality (Galley 1997<sup>167</sup> excluded)**

### 3.2.5.1.2 Effect of single nutrients versus combined micronutrient supplements on infectious complications

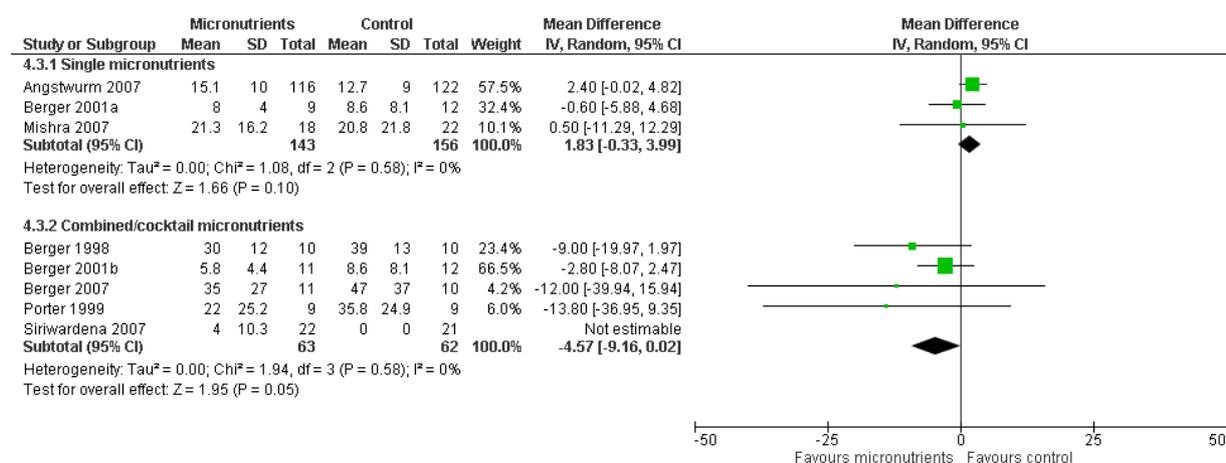
Three of the single nutrient studies<sup>156,159,166</sup> and five of the combined micronutrient studies<sup>159,168,170-172</sup> reported infectious complications as number of patients with infections. When the results of the three studies using single micronutrients were aggregated (n=319), single micronutrients were not associated with a significant difference in infectious complications (RR 1.20, 95% CI 0.81-1.78, I<sup>2</sup>=0%, p=0.36; Figure 3.13). Similarly when the results of the five studies using combined micronutrients were pooled (n=719), combined micronutrients were not associated with a significant difference in infectious complications (RR 0.85, 95% CI 0.60-1.23, I<sup>2</sup>=15%, p=0.39; Figure 3.13).



**Figure 3.13: Effect of single nutrients versus combined micronutrient supplements on infectious complications**

### 3.2.5.1.3 Effect of single nutrients versus combined micronutrient supplements on length of ICU and hospital stay

Three of the single nutrient studies<sup>156,159,169</sup> and five of the combined micronutrient studies<sup>159,160,163,171,175</sup> reported LICU as mean  $\pm$  standard deviations (SD). When the results of the single nutrient studies were pooled (n=299), single nutrients were not associated with a significant difference in LICU (MD 1.83, 95% CI -0.33-3.99,  $I^2=0\%$ ,  $p=0.10$ ; Figure 3.11). On the other hand, aggregating the results of the five combined micronutrient studies (n=125) indicated borderline statistical significance in terms of LICU (MD -4.57, 95% CI -9.16-0.02,  $I^2=0\%$ ,  $p=0.05$ ; Figure 3.14).



**Figure 3.14: Effect of single nutrients versus combined micronutrient supplements on length of ICU stay**

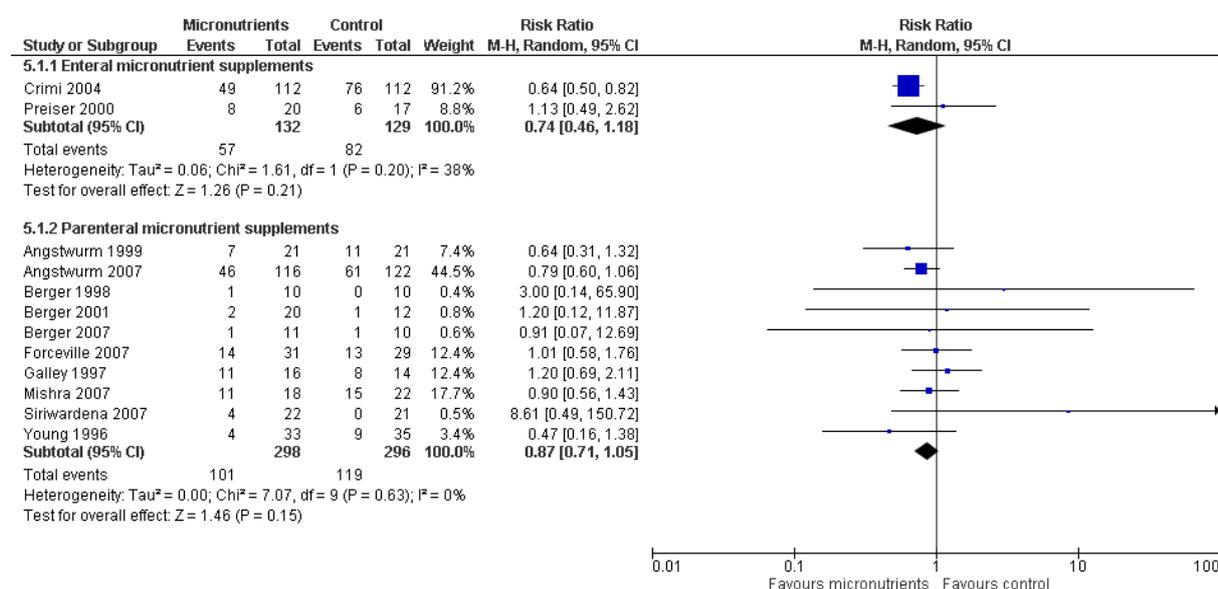
Only one of the single nutrient<sup>159</sup> and four of the combined micronutrient studies<sup>159,163,171,175</sup> reported LOS as mean  $\pm$  standard deviations (SD). Neither the single nutrient study (n=21) nor the studies of combined micronutrients (n=104) had an effect on LOS (MD 18.00, 95% CI -37.53-73.53,  $p=0.53$  and MD -2.92, 95% CI -14.97-9.14,  $I^2=21\%$ ,  $p=0.64$  respectively).

### 3.2.5.2 Parenteral/intravenous versus enteral route micronutrient supplements

Micronutrients were supplied via the parenteral/intravenous route in 11 of the 15 studies,<sup>156,158-160,163,166-169,175,176</sup> via an enteral formula in two of the studies<sup>165,172</sup> and via combined routes of parenteral/intravenous and enteral in a further two studies.<sup>170,171</sup> Studies that used both routes of enteral and parenteral administration<sup>170,171</sup> were excluded from the sub-group analyses of parenteral vs. enteral administration.

### 3.2.5.2.1 Effect of parenteral/intravenous versus enteral route micronutrient supplements on mortality

Ten of the 11 parenteral/intravenous route studies<sup>156,158-160,163,166,167,169,175,176</sup> and both of the enteral route studies<sup>165,172</sup> reported mortality data. When the results of the 11 studies using parenteral micronutrients were aggregated (n=594), there was no significant effect on overall mortality (RR 0.87, 95% CI 0.71-1.05, I<sup>2</sup>=0%, p=0.15; Figure 3.15). Similarly pooled results of the two enteral route studies (n=261) showed no significant effect on overall mortality (RR 0.74, 95% CI 0.46-1.18, I<sup>2</sup>=38%, p=0.21; Figure 3.15).



**Figure 3.15: Effect of enteral versus parenteral micronutrient supplements on overall mortality**

In a sensitivity analysis of the parenteral group the study with the very short intervention duration (6 hours)<sup>167</sup> was excluded, and although the overall estimate of treatment effect did not change appreciably, it did approach significance [adjusted RR without (Galley 1997)<sup>167</sup> 0.83, 95% CI 0.67-1.02, I<sup>2</sup>=0%, p=0.07].

### 3.2.5.2.2 Effect of parenteral/intravenous versus enteral route micronutrient supplements on infectious complications

Four of the parenteral route studies<sup>156,159,166,168</sup> and one of the enteral route studies<sup>172</sup> reported infectious complications as number of patients with infections. Neither the enteral route study (n=37) nor the studies of parenteral micronutrients (n=376) had an

effect on infectious complications (RR 2.55, 95% CI 0.29-22.31,  $p=0.40$  and RR 1.21, 95% CI 0.85-1.72,  $I^2=0\%$ ,  $p=0.30$  respectively).

### **3.2.5.2.3 Effect of parenteral/intravenous versus enteral route micronutrient supplements on length of ICU and hospital stay**

None of the enteral route studies reported on LICU or LOS as mean  $\pm$  standard deviations (SD). Six of the parenteral route studies<sup>156,159,160,163,169,175</sup> reported on LICU whilst three<sup>159,163,175</sup> reported on LOS as mean  $\pm$  standard deviations (SD). Parenteral micronutrients had no significant effect on LICU (MD -0.97, 95% CI -7.12-5.18,  $I^2=39\%$ ,  $p=0.76$ ) or LOS (MD 0.65, 95% CI -15.63-16.92,  $I^2=37\%$ ,  $p=0.94$ ).

### **3.2.5.3 Study quality based on concealment of allocation, intention-to-treat-analysis or level of blinding**

The effect of study quality (including concealment of allocation, intention-to-treat-analysis and level of blinding) on each of the primary outcomes was assessed (Table 3.4). For each of the outcomes only the studies fulfilling the criteria related to a selected study quality domain were included. When only blinded studies were included for the assessment of overall mortality and the results of the relevant nine RCTs were aggregated ( $n=762$ ), these studies were associated with a significant reduction in mortality (RR 0.78, 95% CI 0.65-0.93,  $I^2=8\%$ ,  $p=0.006$ ). When only studies undertaking intention-to-treat analyses were included for the assessment of overall mortality and the results of the relevant 11 RCTs were aggregated ( $n=598$ ), statistical significance was borderline (RR 0.80, 95% CI 0.64-1.01,  $I^2=14\%$ ,  $p=0.06$ ).

**Table 3.4: Effect of study quality on primary outcomes**

Primary Outcomes ↓	Study quality domain assessed			
	Allocation Concealment	Blinding	Intention-to treat (ITT)	All (Allocation Concealment, Blinding and ITT)
<b>Overall mortality</b>	6* 163,159,171,175,165, 166**  n=397*** RR 0.87 95% CI 0.53-1.43 I <sup>2</sup> =38% p=0.58	9 163,159,169,175,176,156, 165,166,172  n=762 RR 0.78 95% CI 0.65-0.93 I <sup>2</sup> =8% <b>p=0.006</b>	11 158,163,159,160,167,169, 171,175,176,165,166  n=598 RR 0.80 95% CI 0.64-1.01 I <sup>2</sup> =14% <b>p=0.06</b>	5 163,159,175,165,166  n=379 RR 0.87 95% CI 0.53-1.43 I <sup>2</sup> =38% p=0.58
<b>Infectious complications</b>	4 159,166,168,171  n=156 RR 1.03 95% CI 0.68-1.55 I <sup>2</sup> =31% p=0.90	5 156, 159,166,168,172  n=413 RR 1.23 95% CI 0.87-1.75 I <sup>2</sup> =0% p=0.24	4 159,166,168,171  n=156 RR 1.03 95% CI 0.68-1.55 I <sup>2</sup> =31% p=0.90	3 159,166,168  n=138 RR 1.24 95% CI 0.84-1.84 I <sup>2</sup> =0% p=0.27
<b>Length of ICU stay</b>	4 163,159,171,175  n=113 MD -3.83 95% CI -8.96-1.30 I <sup>2</sup> =8% p=0.14	5 156,163,159,169,175  n=373 MD -0.38 95% CI -4.49-3.73 I <sup>2</sup> =47% p=0.85	6 163,01,07,169,171,175  n=174 MD -3.13 95% CI -7.23-0.98 I <sup>2</sup> =0% p=0.14	3 163,159,175  n=95 MD -3.67 95% CI -9.86-2.52 I <sup>2</sup> =27% p=0.25
<b>Length of Hospital stay</b>	4 163,159,171,175  n=113 MD -2.00 95% CI -14.23-10.24 I <sup>2</sup> =22% p=0.75	3 163,159,175  n=95 MD 3.00 95% CI -7.50-13.51 I <sup>2</sup> =0% p=0.58	4 163,159,171,175  n=113 MD -2.00 95% CI -14.23-10.24 I <sup>2</sup> =22% p=0.75	3 163,159,175  n=95 MD 3.00 95% CI -7.50-13.51 I <sup>2</sup> =0% p=0.58

\* Indicates number of relevant publications included; \*\* References; \*\*\* Indicates number of participants for the relevant outcome

### 3.3 SECONDARY OBJECTIVES

All 18 included studies provided outcomes related to the secondary objectives.<sup>156,158-160,163-176</sup> All results in this section were obtained from these 18 trials which included 1849 participants.

#### 3.3.1 Doses, Timing and Practice issues

The studies included in the review indicate considerable variability in terms of practices relating to the route of administration, duration and dose of supplementation as well as the type of micronutrients administered singly or in combination (Table 3.5; detailed exposition Appendix 6.6).

**Table 3.5: Timing, doses and practice issues as reported in the various studies**

Study, Year, ID	Route	Timing	Micronutrient(s)	Doses	Maximum dose (/day)	Practice issues
Angstwurm 2007 (S3)	IV	Day 1 <sup>b</sup> - 14	Selenium	1000 µg/day Total: 15 000 µg	1000 µg	Bolus(30 min): day 0 Continuous IV infusion
Angstwurm 2004 & 1999 <sup>a</sup> (S5 & S6 <sup>a</sup> )	IV	Day 1 - 9	Selenium	535 µg: 3 days 285 µg: 3 days 155 µg: 3 days 35 µg: remainder of Rx time	535 µg	Continuous IV infusion
Berger 2001 (S14 <sup>a</sup> & S26)	IV	Day 1 - 5	<i>Se<sup>only</sup> group:</i> Selenium <i>Se<sup>plus</sup> group:</i> Selenium Copper Zinc α-tocopherol	500 µg  500 µg 2.6 mg 13 mg 150 mg	500 µg  500 µg 2.6 mg 13 mg 150 mg	Slow IV infusion  Slow IV infusion  Vit E: Slow injection
Berger 2007 (S15 <sup>a</sup> & S18)	IV	Day 1 - 14 (Burns 20–60% BSA) Day 0 - 21 (Burns >60% BSA)	Selenium Copper Zinc	375 µg 3.75 mg 37.5 mg	375 µg 3.75 mg 37.5 mg	Continuous IV infusion
Berger 1998 (S33)	IV	Day 1 - 8	Selenium Copper Zinc	315 µg 2.5 mg 26.2 mg	315 µg 2.5 mg 26.2 mg	Continuous IV infusion
Cheng 2006 (S52)	IV	Day 1 - 14	<i>B<sub>6</sub>-100 group:</i> Vitamin B6 <i>B<sub>6</sub>-50 group:</i> Vitamin B6	100 mg  50 mg	100 mg	Injection
Crimi 2004 (S54)	EN	Day 1 - 10	Ascorbic acid α-tocopherol	500 mg 400 IU (180 mg)	500 mg 400 IU (180 mg)	Via enteral feeding preparation
Forceville 2007 (S65)	IV	Day 1 - 10	Selenium	4 000 µg: 1 day 1 000 µg: 9 days	4 000 µg	Continuous IV infusion
Galley 1997 (S68)	IV	0 - 6 hours	Ascorbic acid α-tocopherol	1000 mg 400 mg	1000 mg 400 mg	Bolus doses
Maderazo 1991 (S92)	IV	Day 1 - 7	Ascorbic acid α-tocopherol	200 mg, then ↑ 500 mg 50 mg	500 mg 50 mg	Continuous IV infusion
Mishra 2007 (S103)	IV	Day 1 - 9 Day 9 – discharged (lower dose)	Selenium	474 µg: 3 days 316 µg: 3 days 158 µg: 3 days 31.6 µg: remainder of Rx time	474 µg	Continuous IV infusion

Study, Year, ID	Route	Timing	Micronutrient(s)	Doses	Maximum dose (/day)	Practice issues
Nathens 2002 (S107)	IV and EN	Day 1 - shorter duration of ICU admission or 28 days	Ascorbic acid  $\alpha$ -tocopherol (dl- $\alpha$ -tocopheryl acetate)	1000 mg q8h (3000 mg/day) 1000 IU (450 mg) q8h (3000 IU/1350 mg/day)	3000 mg  3000 IU / 1350 mg	IV infusion  Via naso- or orogastric tube
Porter 1999 (S115)	IV and EN or PO	Day 1 - 7	Selenium  Ascorbic acid  $\alpha$ -tocopherol	50 $\mu$ g q6h (200 $\mu$ g/day) 100 mg q8h (300 mg/day) 400 IU (268 mg) q8h (1200 IU/804mg/day)	200 $\mu$ g  300 mg  1200 IU / 804 mg	Se: IV infusion  AA/ $\alpha$ -T: PO or via nasogastric tube
Preiser 2000 (S118)	EN	Day 1 - 7	Vitamin A  [ $\beta$ -carotene]  Ascorbic acid  $\alpha$ -tocopherol	133 $\mu$ g/100 ml ( $\pm$ 1L/day = 1330 $\mu$ g/day) [66.7 $\mu$ g/100 ml ( $\pm$ 1L/day = 667 $\mu$ g/day)] 13.3 mg/100 ml ( $\pm$ 1L/day = 133 mg/day) 4.94 mg/100 ml ( $\pm$ 1L/day = 49.4 mg/day)	1330 $\mu$ g  [667 $\mu$ g]  133 mg  49.4 mg	Via enteral feeding preparation
Rock 1997 (S124)	EN or PO	Day 1 - 21	$\beta$ -carotene	30 mg	30 mg	Capsules: contents mixed in 30ml enteral formula / meal
Rümelin 2005 (S128)	IV	Day 2 - 3 postoperatively	Ascorbic acid	<34.1 $\mu$ mol/l: 2000 mg $\leq$ 56.8 $\mu$ mol/l: 1000 mg $\leq$ 68.2 $\mu$ mol/l: 500 mg >68.2 $\mu$ mol/l: 0 mg	2000 mg	IV infusion
Siriwardena 2007 (S135)	IV	Day 1 - 7	Selenium  Ascorbic acid	1000 $\mu$ g: 1 day 400 $\mu$ g: 1 day 200 $\mu$ g: 5 days 2000 mg: 2 days 1000 mg: 5 days	1000 $\mu$ g  2000 mg	Continuous IV infusion
Young 1996 (S146)	IV then PO	Day 1 - 15 (PN)  Then orally till 3 months after injury	Zinc	12 mg: 15 days (PN)  168 mg zinc gluconate, 22 mg elemental zinc: day 16 till 3 months after injury	12 mg	Via TPN solution  Oral tablet

$\alpha$ : Indicates the major publication for the study; b: Referring to first day of study inclusion (usually within 48-72 hr of ICU admission)

$\alpha$ -T:  $\alpha$ -tocopherol; AA: Ascorbic acid; BSA: Body surface area; EN: Enteral nutrition; ICU: Intensive care unit; IV: Intravenous; PO: Per os; Rx: Treatment; Se: Selenium; (T)PN: (Total) parenteral nutrition; Vit: Vitamin

### 3.3.1.1 Route of administration

Micronutrients were supplied via the parenteral/intravenous route in the majority of studies (13 of 18) included for the secondary objectives,<sup>156,158-160,163,164,166-169,174-176</sup> via combined routes of parenteral/intravenous and enteral in two studies,<sup>170,171</sup> via an enteral formula in a further two studies,<sup>165,172</sup> and via an enteral formula or orally in one study.<sup>173</sup>

### 3.3.1.2 Timing

In all the trials except one,<sup>174</sup> micronutrient supplementation was initiated on the first day of study inclusion (the latter usually being within 24-72 hours of ICU admission). Micronutrient intervention lasted anything from 6 hours to discharge, with the average duration of intervention (excluding maintenance doses) being 10.8 (7.02) days (Figure 3.16).

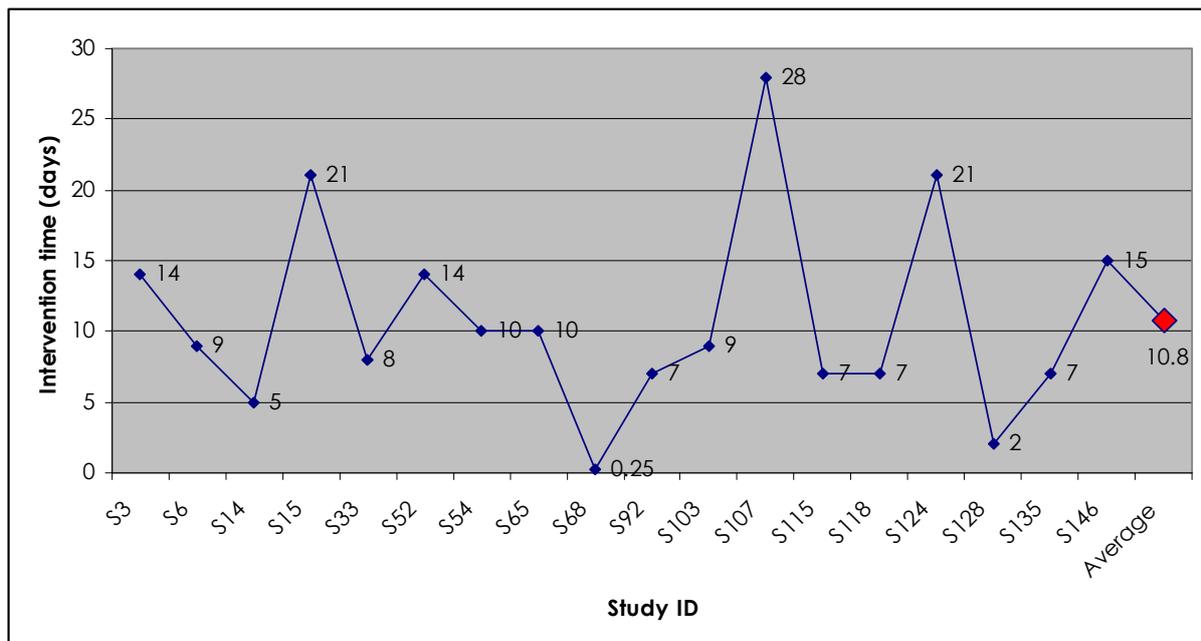
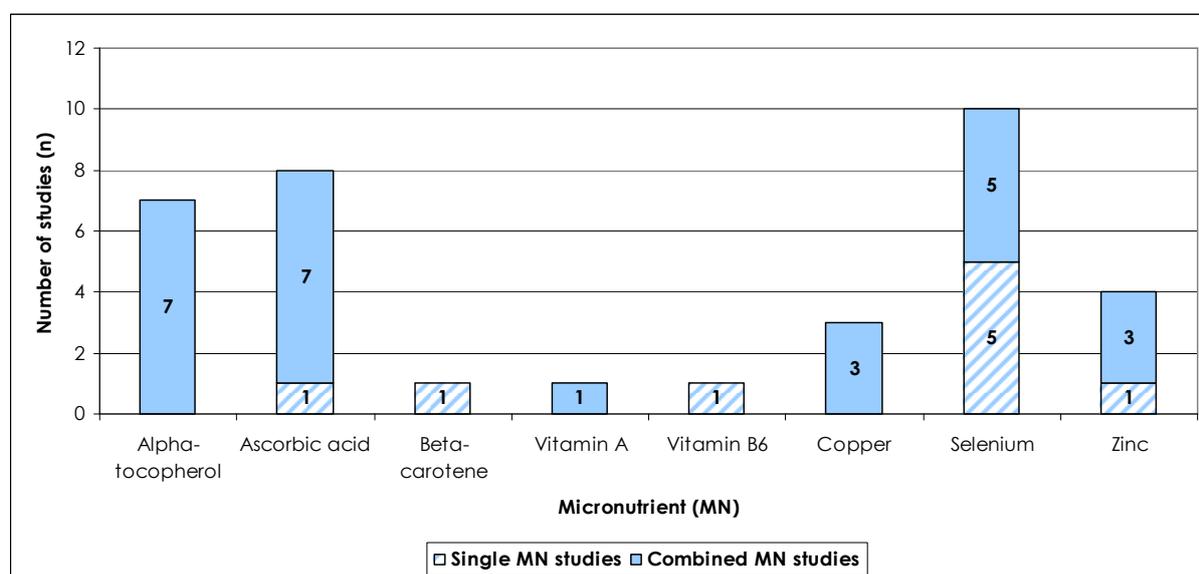


Figure 3.16: Study intervention duration

### 3.3.1.3 Micronutrients and doses

Nine of the trials used single micronutrients (including the single nutrient intervention group of Berger 2001),<sup>156,158,159,164,166,169,173,174,176</sup> whilst ten trials provided combined micronutrient supplements (including the combined micronutrient intervention group of Berger 2001).<sup>159,160,163,165,167,168,170-172,175</sup> Selenium was the most commonly used single nutrient (n=5), whilst  $\alpha$ -tocopherol (n=7) and ascorbic acid (n=7) were the most

commonly used micronutrients in studies of combined supplements (Figure 3.17). The micronutrient doses used in the various trials varied greatly (Table 3.5 and 3.6).



**Figure 3.17: Use of specific micronutrients in single and combined supplement studies**  
MN: Micronutrient

**Table 3.6: Micronutrient dose ranges used in intervention trials (IV: Intravenous)**

Micronutrient	Number of studies	Doses in critical care
α-tocopherol	3	50 – 400 mg (IV)
	4	50 – 1350 mg (Enteral)
Ascorbic acid	5	500 mg – 3 g (IV)
	3	133 – 500 mg (Enteral)
β-carotene	1	30 mg (Enteral)
Vitamin A	1	1330 µg (Enteral)
Vitamin B <sub>6</sub>	1	100 mg (IV)
Copper	3	2.5 – 3.75 mg (IV)
Selenium	10	200 – 4000 µg (IV)
Zinc	4	12 – 37.5 mg (IV)

### **3.3.2 Micronutrient Status / Morbidity / Course of the APR / Oxidative Stress**

Micronutrient status, morbidity, course of the APR and measures of oxidative stress in the included studies were variably and sparsely reported (e.g. full data tables versus text reporting in varying degrees of detail) to such an extent that proper data aggregation was not possible. As such only descriptive tables, summarizing the main findings / conclusions of the various studies were included for these outcomes.

#### **3.3.2.1 Micronutrient status**

Only two<sup>166,171</sup> of the 18 trials did not provide information on the micronutrient status of the patients after supplementation. The majority of trials (11 of 16)<sup>156,158-160,164,165,167,169,172-174</sup> reported significant increases in micronutrient concentrations (for some or all of the micronutrients measured) after micronutrient supplementation (Table 3.7). Two trials simply reported an increase (correction/normalization) of micronutrient concentrations in experimental groups,<sup>168,170</sup> two trials reported increases in the experimental groups with no significant differences between groups<sup>175,176</sup> and one trial reported a significant decrease in the control group (whilst the micronutrient supplemented group maintained normal levels).<sup>163</sup>

**Table 3.7: Micronutrient status of patients after supplementation**

Study, Year, ID	Micronutrients measured	Main findings / conclusions	Simplified outcome
Angstwurm 2007 (S3)	Selenium	Whole blood Se concentrations were within the upper normal range during Se treatment, whereas they remained significantly low in the placebo group ( $p \leq 0.001$ ).	Significant $\uparrow$ in Rx group
Angstwurm 2004 & 1999 <sup>a</sup> (S5 & S6 <sup>a</sup> )	Selenium	In Rx group, serum Se levels normalized within 3 days, whereas in controls it remained significantly low ( $p \leq 0.001$ ).	Significant $\uparrow$ in Rx group
Berger 2001 (S14 <sup>a</sup> & S26)	Selenium Copper Zinc $\alpha$ -tocopherol	Plasma Se and tocopherol concentrations were low on admission, but $\uparrow$ significantly ( $p=0.001$ ) with supplementation; Cu/Zn: no statistically significant differences.	Significant $\uparrow$ in Rx group: Se, $\alpha$ -tocopherol  NS for Cu, Zn
Berger 2007 (S15a <sup>a</sup> & S18)	Selenium Copper Zinc $\alpha$ -tocopherol Retinol	Plasma Cu and Se concentrations were significantly higher after day 5 in the Rx group ( $p=0.013$ and $p<0.0001$ respectively); There were no significant differences in plasma Zn, retinol and tocopherol between the groups.	Significant $\uparrow$ in Rx group: Cu, Se  NS for Zn, retinol, $\alpha$ -tocopherol
Berger 1998 (S33)	Selenium Copper Zinc	Mean plasma Cu and Zn concentrations were below normal until days 20 and 15, respectively (NS); Plasma Se remained normal for Rx group but $\downarrow$ for control group ( $p<0.05$ on days 1 and 5).	Significant $\downarrow$ in control group: Se  NS for Cu, Zn
Cheng 2006 (S52)	Plasma pyridoxal 5'-phosphate	Plasma PLP concentrations significantly $\uparrow$ in two Rx groups ( $p<0.05$ ).	Significant $\uparrow$ in Rx groups
Crimi 2004 (S54)	Plasma and LDL-bound Vitamin E	Significant $\uparrow$ in the concentration of plasma and LDL-bound vitamin E ( $p<0.01$ for both comparisons).	Significant $\uparrow$ in Rx groups
Galley 1997 (S68)	Vitamin C	Vitamin C concentrations significantly $\uparrow$ in patients in the Rx group ( $p=0.0002$ ).	Significant $\uparrow$ in Rx groups
Maderazo 1991 (S92)	Ascorbic acid $\alpha$ -tocopherol	Control group: AA & $\alpha$ -tocopherol remained depressed during study period; Rx group: AA & $\alpha$ -tocopherol inadequacies corrected to within 1 SD of normal mean concentrations ( $p$ -values not available).	$\uparrow$ (correction) in Rx group: AA, $\alpha$ -tocopherol
Mishra 2007 (S103)	Selenium	In the Rx group, plasma Se $\uparrow$ by day 3 and 7 ( $p=0.0001$ ) and day 14 ( $p=0.02$ ) as compared to control group.	Significant $\uparrow$ in Rx groups
Nathens 2002 (S107)	Ascorbic acid $\alpha$ -tocopherol	AA concentrations remained low (3 weeks) in control group; Plasma AA concentrations normalized within 1 day of admission and met or exceeded the upper limit of normal in the Rx group; Plasma $\alpha$ -tocopherol concentrations were comparable in both groups at baseline and diverged by 3 days of treatment, reaching supranormal levels by day 5.	$\uparrow$ (correction) in Rx group: AA, $\alpha$ -tocopherol

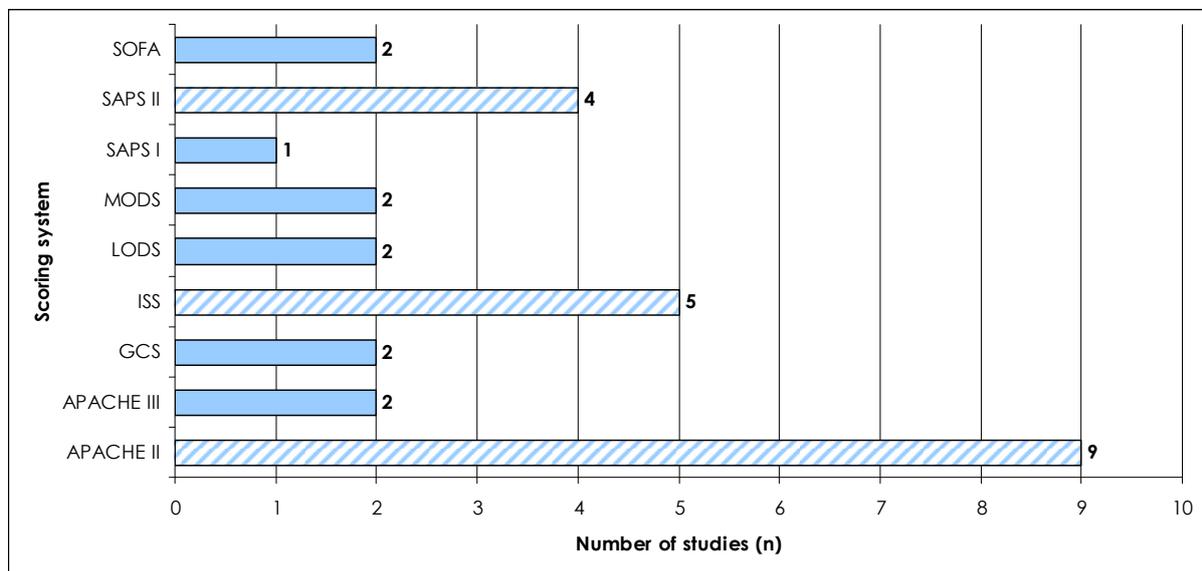
Study, Year, ID	Micronutrients measured	Main findings / conclusions	Simplified outcome
Preiser 2000 (S118)	Vitamin A β-carotene α-tocopherol	Significant ↑: concentration of plasma β- carotene (p<0.01) and plasma LDL-bound α-tocopherol (p<0.05); Plasma Vitamin A: NS.	Significant ↑ in Rx group: β-carotene, α-tocopherol  NS for Vit A
Rock 1997 (S124)	β-carotene α-tocopherol Vitamin C Retinol	Vit C, α-tocopherol and retinol concentrations low at baseline, but levels ↑ significantly over the study period in Rx and control groups (p<0.05); Plasma β-carotene concentration ↑ significantly (p<0.003) across weeks in Rx but not control groups.	Significant ↑ in Rx group: β-carotene  Significant ↑ in Rx and control groups: Vit C, α-tocopherol, retinol
Rümelin 2005 (S128)	Ascorbic acid	At end of the study period, the AA concentration in plasma was significantly (p≤0.001) different between groups.	Significant ↑ in Rx group
Siriwardena 2007 (S135)	Ascorbic acid Selenium	Relative serum levels of antioxidants rose during the course of the trial; Relative to the control group, Rx group antioxidant levels rose; NS between groups AA (p=0.27) & Se (p=0.28).	↑ in Rx group but NS between groups
Young 1996 (S146)	Zinc	NS difference between groups in mean serum Zn concentrations over 28-day study period; S-Zn concentrations depressed at the beginning of study but normalized by day 10 in both groups.	↑ (normalization) in Rx and control groups but NS between groups

α: Indicates the major publication for the study

↑: Increase; ↓: Decrease; AA: Ascorbic acid; Cu: Copper; LDL: Low density lipoprotein; NS: Not significant; PLP: Plasma pyridoxal 5'-phosphate; Rx: Treatment / Experimental; Se: Selenium; SD: Standard deviation; Vit: Vitamin; Zn: Zinc

### 3.3.2.2 Morbidity

Various severity of illness scoring systems (indicating patient morbidity) were utilized in all the included studies, except one,<sup>173</sup> to describe patient populations at baseline. These scoring systems included APACHE (Acute Physiology and Chronic Health Evaluation) II and III, GCS (Glasgow Coma Scale), ISS (Injury Severity Score), LODS (Logistic Organ Dysfunction Score), MODS (Multiple Organ Dysfunction Score), SAPS (Simplified Acute Physiology Score) I and II and SOFA (Sequential Organ Failure Assessment) score. Many of the studies made use of more than one scoring system, with APACHE II (n=9),<sup>158,159,163,164,167,170</sup> ISS (n=5)<sup>159,165,168,170,171</sup> and SAPS II (n=4)<sup>160,166,172,174</sup> most often used (Figure 3.18).



**Figure 3.18: Frequency of use of scoring systems to describe patient populations at baseline**

Although the majority of studies used scoring systems to describe patient populations at baseline, only eight studies reported the scoring systems as study endpoints.<sup>156,158,164,165,169,170,174,175</sup> Outcomes varied and included: 1) significant decrease in the experimental group,<sup>164,169,170</sup> 2) significant decrease in both the experimental and control groups,<sup>156,175</sup> 3) no difference (not significant) between groups<sup>156,165,169,174,175</sup> and 4) significant difference between experimental and control groups (favouring experimental group)<sup>158</sup> (Table 3.8).

**Table 3.8:** Morbidity of patients after supplementation

Study, Year, ID	Scoring system	Main findings / conclusions	Simplified outcome
Angstwurm 2007 (S3)	APACHE III	↓ from day 1 to day 28 in the Rx group and control groups (p=0.0002 for both); NS between groups.	Significant ↓ in both groups; NS between groups.
	LODS	Similar in both groups (NS).	No difference between groups.
Angstwurm 2004 & 1999 <sup>a</sup> (S5 & S6 <sup>a</sup> )	APACHE III	Significant ↓ in Rx group vs control group [days 7 (p=0.019) and 14 (p=0.041)].	Significant ↓ in Rx group vs control group.
Cheng 2006 (S52)	APACHE II	Significantly ↓ by day 14 in B <sub>6</sub> -50 group (Rx group 1) only (p<0.05); NS for control and B <sub>6</sub> -100 group (Rx group 2).	Significant ↓ in 1 Rx group only.
Crimi 2004 (S54)	SAPS ISS	NS difference between groups in the ITT analysis for SAPS or ISS.	NS differences between groups for SAPS and ISS.
Mishra 2007 (S103)	SOFA	↓ in both groups; ↓ significantly in Rx group by day 7 and 14 as compared to day 0 (p=0.006: day 0-7, p<0.006: day 0-14) but not in control group (p=0.08: day 0-7, p=0.09: day 0-14); NS difference between the Rx and control group on any day (day 7, p=0.59; day 14, p=0.40).	Significant ↓ in Rx group; NS between groups.
Nathens 2002 (S107)	MODS	Significantly lower in the Rx group (MD 0.9, 95% CI 0.50–1.06).	Significant ↓ in Rx group.
Rümelin 2005 (S128)	APACHE II SAPS II	Both scores on the second and third postoperative days were not different between groups.	NS differences between groups for both.
Siriwardena 2007 (S135)	APACHE II	↓ in both groups as expected but no difference between groups after first 7 days of Rx.	↓ in both groups but NS between groups.
	MODS	Analysis at day 7 revealed a trend towards more MOD in patients on active Rx than control (difference at 7 days, p=0.093).	NS between groups (trend toward more MOD in Rx group).

a: Indicates the major publication for the study

↓: Decrease; APACHE: Acute Physiology and Chronic Health Evaluation; GCS: Glasgow Coma Scale; ISS: Injury Severity Score; ITT: Intention-to-treat; LODS: Logistic Organ Dysfunction Score; MOD: Multiple Organ Dysfunction; MODS: Multiple Organ Dysfunction Score; NS: Not significant; Rx: Treatment / Experimental; SAPS: Simplified Acute Physiology Score; SOFA: Sequential Organ Failure Assessment score

### 3.3.2.3 Course of APR

A further secondary objective was to describe the APR as judged by cytokine levels, WBC and differential WBC count, and other inflammatory markers (specifically CRP). Although reporting was highly variable and sparse, eight trials (of 18) provided information regarding the course of the APR (Table 3.9). Three of these trials reported on cytokine levels,<sup>160,163,170</sup> four trials reported on WBC and/or differential WBC count,<sup>159,163,164,170</sup> and seven trials reported on inflammatory markers (specifically CRP).<sup>156,159,160,163,164,169,175</sup> In broad terms, no consistent patterns of alterations in the profiles of these parameters emerged from the studies included in the review, except perhaps for CRP.

*Cytokine levels:* The three studies provided information on IL-1, IL-6, IL-8, IL-10 and TNF- $\alpha$ . Two studies found no significant differences between groups at the end of the experimental period,<sup>160,170</sup> whilst the remaining study only showed a significant difference on day 1 after study commencement (in favour of the experimental group)<sup>163</sup> (Table 3.9).

*WBC and/or differential WBC count:* One study found significant increases in various types of WBC in both its experimental groups,<sup>164</sup> one study found a significant increase in total leukocyte count at one point in time when compared to control (not significant at end of study period),<sup>163</sup> one study showed increases but provided no further data<sup>159</sup> and the final study found no difference between groups<sup>170</sup> (Table 3.9).

*C-reactive protein:* Of the seven studies, three reported decreasing CRP levels but no significant differences between groups,<sup>156,160,175</sup> two studies reported significant reductions in both their experimental and control groups,<sup>164,169</sup> one study found a significant decrease (experimental group) at one point in time (not significant at end of study period)<sup>163</sup> and one study showed decreases but provided no further data<sup>159</sup> (Table 3.9).

**Table 3.9: The course of the Acute Phase Response in relation to micronutrient supplementation**

Study, Year, ID	Cytokine levels		WBC and/or differential WBC count		C-Reactive Protein	
	Main findings	Simplified outcome	Main findings	Simplified outcome	Main findings	Simplified outcome
Angstwurm 2007 (S3)					↓ in both groups, but NS between groups.	↓ levels but NS between groups.
Berger 2001 (S14 <sup>a</sup> & S26)			Leukocytes ↑ in all groups: table provided: no statistics, discussion or comparisons.	↑ in all groups (no further data).	↓ in all groups; table provided: no statistics, discussion or comparisons.	↓ in all groups (no further data).
Berger 2007 (S15 <sup>a</sup> & S18)	NS differences in IL-6 and IL-10 between groups.	NS between groups.			NS differences between groups.	↓ levels but NS between groups.
Berger 1998 (S33)	Difference between groups was significant on day 1 for IL-6 (p<0.001) (values ↑ in control group).	Significant difference on day 1 (in favour of experimental group).	Total leukocyte counts tended to be ↑ in Rx group due to higher neutrophil counts [only significantly different between groups on day 15 (p<0.05)].	Significant difference on day 15 (in favour of experimental group).	Difference between groups significant on day 2 (p<0.05) (values ↑ in control group).	Significant difference on day 2 (in favour of experimental group).
Cheng 2006 (S52)			T-lymphocyte, T-helper cell nrs, % T-suppressor cells significantly ↑ in B <sub>6</sub> -50 group: D14; Total lymphocyte count, T-helper, T-suppressor cell nrs, % T-lymphocyte cells, T-suppressors significantly ↑ in B <sub>6</sub> -100 group: D14; p<0.05 for both; NS changes: control group (14 days).	Significant ↑ in various types of WBC in both Rx groups.	Significant ↓ in control and B <sub>6</sub> -50 groups (Rx group 1) by day 14 (p<0.05); NS for B <sub>6</sub> -100 group (Rx group 2).	Significant ↓ in 1 experimental and control group.

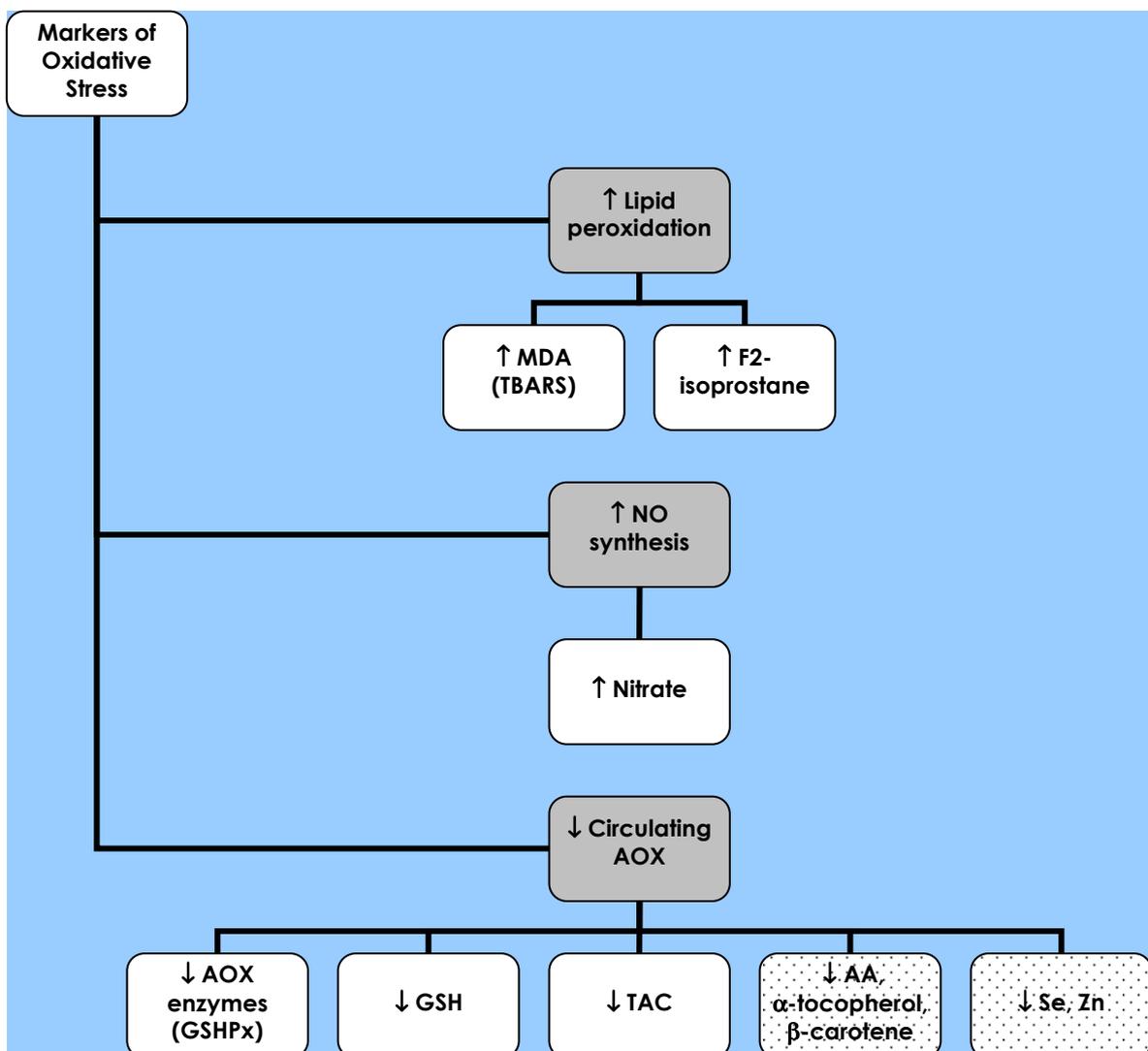
Study, Year, ID	Cytokine levels		WBC and/or differential WBC count		C-Reactive Protein	
	Main findings	Simplified outcome	Main findings	Simplified outcome	Main findings	Simplified outcome
Mishra 2007 (S103)					Significant ↓ by day 14 (p=0.003 in Rx group, p<0.0001 in control group).	Significant ↓ in both groups.
Nathens 2002 (S107)	↓ concentrations of TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 in Rx group, similar between groups for IL-8; NS differences between any groups (p=0.27 / 0.26 / 0.64 / 0.81 respectively).	↓ concentrations in Rx group but NS between groups.	WBC: No difference between Rx and control group (p=0.42).	NS between groups.		
Siriwardena 2007 (S135)					No difference between groups at end of study period (p=0.61).	↓ levels but NS between groups.

$\alpha$ : Indicates the major publication for the study

↑: Increase; ↓: Decrease; D: Day; IL: Interleukin; Nrs: Numbers; NS: Not significant; Rx: Treatment / Experimental; WBC: White blood cell

### 3.3.2.4 Oxidative stress

Ten trials<sup>156,158-160,165,167,168,170,172,175</sup> reported on the changes observed in the level of oxidative stress using an array of plasma variables as markers (Figure 3.19; Table 3.10). These plasma variables include malonyldialdehyde acid (thiobarbituric acid reactive substances) [MDA (TBARS)], F2-isoprostanes, nitrates, selenoenzyme glutathione peroxidase (GSHPx), glutathione (GSH), total antioxidant capacity (TAC) and circulating antioxidants (including ascorbic acid,  $\alpha$ -tocopherol,  $\beta$ -carotene, selenium and zinc which were reported under 3.3.2.1). In broad terms, no consistent patterns of alterations in the profiles of these parameters emerged from the studies included in the review.



**Figure 3.19: Summary of oxidative stress signals and corresponding plasma variables observed in the included trials**

AA: Ascorbic acid; AOX: Antioxidants; GSH: Glutathione; GSHPx: Selenoenzyme glutathione peroxidase; MDA: Malonyldialdehyde acid; NO: Nitric oxide; Se: Selenium; TAC: Total antioxidant capacity; TBARS: Thiobarbituric acid reactive substances; Zn: Zinc   Reported under 3.3.2.1.

Five trials provided information on GSHPx,<sup>156,158-160,169</sup> three trials reported on MDA (TBARS),<sup>165,167,172</sup> three on F2-isoprostanes,<sup>165,169,170</sup> two on TAC<sup>159,167</sup> and one each on GSH/GSSH (glutathione / glutathione disulphide) ratio<sup>175</sup> and nitrates.<sup>167</sup>

*GSHPx:* Studies found significant increases in the experimental group itself,<sup>158-160,169</sup> significant increases in the experimental group when compared to control<sup>156,169</sup> and one study found no change in any group for RBC (Red blood cell) GSHPx<sup>169</sup> (Table 3.10).

*GSH/GSSG (Glutathione/Glutathione disulphide) ratio:* One study reported no difference between groups<sup>175</sup> (Table 3.10).

*TAC:* One study reported an early significant decrease in the experimental group<sup>159</sup> whilst the other study reported that TAC was unaffected by supplementation<sup>167</sup> (Table 3.10).

*MDA (TBARS):* One study reported a significant decrease in the experimental group,<sup>165</sup> with two studies finding no difference between groups.<sup>167,172</sup> Two studies<sup>165,172</sup> did report improved LDL (low density lipoprotein) resistance to oxidative stress in their experimental groups (Table 3.10).

*F2-isoprostanes:* One study found a significant decrease in the experimental group,<sup>165</sup> another study reported a decrease that did not reach significance<sup>170</sup> and the final study<sup>169</sup> reported no significant difference in any group or between groups (Table 3.10).

*Nitrates:* The only study<sup>167</sup> to report on nitrates indicated a significant increase in the experimental group (Table 3.10).

**Table 3.10: Oxidative stress markers and outcome in relation to micronutrient supplementation**

Study, Year, ID	Plasma variable	Main findings / conclusions	Simplified outcome
Angstwurm 2007 (S3)	GPx-3	Activity significantly ↑ in Rx group compared with control group (p<0.001).	Significantly ↑ in Rx group vs control.
Angstwurm 2004 & 1999 <sup>a</sup> (S5 & S6 <sup>a</sup> )	GSHPx	Significant ↑ (p<0.001) in Rx group vs. significantly low or ↓ in control group.	Significantly ↑ in Rx group; significantly low in control group.
Berger 2001 (S14 <sup>a</sup> & S26)	TAC	Early significant fall (p<0.002) in the Rx groups.	Early significant ↓ in Rx group.
	GSHPx	Plasma GSHPx activity ↑ significantly between days 2 and 5 with supplementation (p=0.02), but erythrocyte enzyme activity unaffected.	Significantly ↑ in Rx group.
Berger 2007 (S15 <sup>a</sup> & S18)	GSHPx	Concentrations significantly higher after D 5 in Rx group; time group interaction was highly significant (p<0.0001); Erythrocyte GSHPx did not ↑.	Significantly ↑ in Rx group.
Crimi 2004 (S54)	Lipid peroxidation: MDA (TBARS) F2α isoprostanes	Plasma TBARS and isoprostanes significantly ↓ after intervention (p<0.01 for both comparisons). AOX improved LDL resistance to oxidative stress by approximately 30% (p<0.04).	Significantly ↓ in Rx group. Improved LDL resistance (Rx group)
Galley 1997 (S68)	TAC	Unaffected by supplementation (NS).	Unaffected by supplementation.
	MDA	Lipid peroxides and MDA elevated in all patients but did not ↑ further in Rx group.	No difference between groups.
	Nitrates	Plasma total nitrate ↑ (p=0.007) in the Rx group.	Significant ↑ in Rx group.
Mishra 2007 (S103)	Plasma GSHPx	Plasma GSHPx significantly ↑ in the Rx group by D 3 and 7 as compared to the control group (p=0.01). Plasma GSHPx in the Rx group was significantly higher at D 14 as compared to D 0 (p< 0.0001) but there was no significant ↑ in plasma GSHPx by D 14 in the control group.	Significantly ↑ in Rx group (end of study period) and significantly ↑ vs control (NS for control group).
	RBC GSHPx	There was no change in either group.	No change in any group.
	F2 isoprostanes	NS difference in oxidative status between 2 groups on any D as well as no significant difference noticed by D 14 in either group as compared to D 0.	NS difference in any group or between groups.
Nathens 2002 (S107)	F2α isoprostanes	Median levels appeared to be lower in patients receiving AOX supplementation but NS (p=0.5).	↓s in Rx group but NS.
Preiser 2000 (S118)	Lipid peroxidation: MDA (TBARS)	Improved LDL resistance to oxidative stress by 21±4% (p<0.05). No such change was observed in the control group. No change in total lipid peroxidation, estimated by the TBARS plasma concentration (MDA) (NS).	Improved LDL resistance (Rx group). MDA (TBARS): no change.
Siriwardena 2007 (S135)	GSH/GSSG ratio	No difference between groups at end of study period (p=0.51).	No difference between groups.

a: Indicates the major publication for the study

↑: Increase; ↓: Decrease; AOX: Antioxidants; D: Day; GSH: Glutathione; GSHPx: Selenoenzyme glutathione peroxidase; GSSG: Glutathione disulphide; LDL: Low density lipoprotein; MDA: Malonyldialdehyde acid; NS: Not significant; RBC: Red blood cell; Rx: Treatment / Experimental; TAC: Total antioxidant capacity; TBARS: Thiobarbituric acid reactive substances

## **CHAPTER 4: DISCUSSION**

This systematic review substantially builds on the initial very important review by Heyland et al.<sup>130</sup> by including 7 new trials (for the primary outcomes and meta-analysis), including the first four multi-centre randomized controlled trials in this area of research<sup>156,165,166,175</sup> and a total of 1714 participants (an almost two-fold increase to the 886 participants included in the Heyland et al.<sup>130</sup> review). This updated review indicates and supports previous findings<sup>130</sup> that micronutrient supplementation in the critically ill may be associated with a reduction in overall mortality, and specifically 28 day mortality. With the exception of one trial that warrants caution, micronutrient supplementation was not associated with adverse / deleterious effects. The present review found no significant effect for micronutrient supplementation on infectious complications, was the first to report on LICU and LOS and found that these outcomes were unaffected by micronutrient supplementation. The secondary outcomes confirmed that timing, duration and dosing are key factors to ensure optimal clinical benefit.

The literature consistently indicates that there exists an association between oxidative stress and clinical outcome in critically ill patients.<sup>177-180</sup> Micronutrient supplementation has shown to improve antioxidant capacity as demonstrated by increased activity of GSHPx.<sup>156,158-160,169</sup> However, the micronutrient requirements of the critically ill remain unclear with practitioners uncertain what practices to implement based on the available evidence. Many of the initial smaller trials did not reach definitive conclusions, with only a handful of larger trials now starting to see the light. Furthermore, working with patients in a critical care setting necessitates caution and with that create the fear of “doing more harm than good” (i.e. the potential for creating a pro-oxidant microenvironment<sup>41,129</sup> with deleterious patient outcomes) further complicating decision-making on behalf of the nutrition support practitioner.

#### **4.1 PRIMARY OUTCOMES**

With the exception of three larger trials,<sup>156,165,170</sup> the majority of trials included in this systematic review were still relatively small (<n=100). Well defined patient populations were included in the various trials (i.e. general trauma, SIRS, burns, severe acute pancreatitis, head injury and coronary critical care), though heterogenic between groups, with micronutrient depletion and high oxidative stress states in general being

common among the various groups. Due to the nature of injuries / conditions the vast majority of trials recruited more men than women.

*Mortality:* This updated systematic review found a statistically significant reduction in overall mortality associated with micronutrient supplementation, against the background of best medical practice and adequate nutritional support. This finding is in line with the Heyland et al. systematic review who similarly found a significant reduction in mortality ( $p=0.03$ ).<sup>130</sup> Caution should however be exercised when directly interpreting the results of this review as an exploratory funnel plot analysis indicated that publication or other sources of bias (e.g. with respect to study size and poor methodological design of small studies) could, to some extent, account for some of the apparent benefit observed with respect to overall mortality. It could be argued that the exclusion of foreign language studies in this systematic review (language bias) might have contributed to this observed bias (Appendix 6.8). A sensitivity analysis (excluding one study with a very short, i.e. 6 hour intervention) still indicated a significant reduction in overall mortality. When broken down into categories, this systematic review found no significant effect for micronutrient supplements on hospital and ICU mortality (the latter only reported in two trials). On the other hand, when the results of the relevant six trials that reported on 28 day mortality were aggregated ( $n=1194$ ), micronutrient supplementation was associated with a significant reduction in 28 day mortality (not reported on in the Heyland et al.<sup>130</sup> review). No evidence of statistical heterogeneity was present and a subsequent funnel plot analysis indicated that there is little or no indication that a publication or other bias accounts for the apparent benefit observed. It should be noted that tests for funnel plot asymmetry are usually recommended when there are at least 10 studies included in the analysis, because when there are fewer studies the power of the tests can be too low to distinguish chance from real asymmetry.<sup>11</sup> This should be borne in mind when interpreting the funnel plot analysis for 28 day mortality which included only six studies.

Importantly, the vast majority of trials reported no adverse / deleterious effects of micronutrient administration, with the exception of one recent trial that warrants mention.<sup>175</sup> The study by Siriwardena et al.,<sup>175</sup> the only included study conducted in patients with severe acute pancreatitis, indicated a trend towards worse outcome in

the treatment arm of the study (although results were not statistically significant and not demonstrably treatment related). The authors acknowledge that the study was underpowered with risk of type II error, with deaths occurring in patients with more severe disease and multiple organ failure. The authors indicate that data suggest that intravenous antioxidants might be harmful in patients with baseline organ dysfunction at two or more organ sites (however this in itself could be interpreted as a chance finding). However, the outcome of this study warrants caution, at least in patients with severe acute pancreatitis. In general terms, practitioners will be well served by remembering "more is not necessarily better". Micronutrients may have dose-response curves with toxicity risk at high levels of intake. Zinc toxicity has been reported with doses of over 50 mg per day<sup>181</sup> and an upper limit of intake of selenium in the diet has been set at 400 µg (5 µg/kg) per day (although a no observable effect level of 800 µg per day has been proposed).<sup>145</sup> An upper limit for safe short-term intravenous supplementation of 750-1000 µg selenite per day in the critically ill has been suggested<sup>182</sup> with a dose of 800 µg of selenium per day given via the combined IV and enteral route documented to be safe in a dose-finding study.<sup>183</sup> Results of a meta-analysis of 19 RCTs in the community suggest that high dosages of vitamin E increase risk of all-cause mortality, and this dose-dependant increase begins at 150 IU/day.<sup>184</sup> It is not clear to what extent all these considerations apply to the critically ill, but certainly should warrant a word of caution. More dose-finding trials are therefore required (as was recently published by Heyland et al.<sup>183</sup>) to establish optimal doses.

*Infectious complications:* Micronutrient supplementation in this systematic review was not associated with a reduction in infectious complications, similar to the findings of Heyland et al.<sup>130</sup> This finding, now confirmed again in this updated review could possibly indicate that the mortality effect observed was mediated by other mechanisms (e.g. improved organ function). It should be noted that the results of four trials could not be included for analysis (see results section), with two of these trials (in burns patients) finding significant reductions in number of infectious complications per patient in the micronutrient supplemented groups<sup>160,163</sup> and the other two trials finding no difference.<sup>169,165</sup>

*LICU and LOS:* Length of ICU stay (LICU) and length of stay in hospital (LOS) were not affected by micronutrient supplementation in this systematic review (not reported in Heyland et al.<sup>130</sup> due to sparsely and variably recorded data at the time). Four trials for both outcomes could not be included in the analyses (see results section) but it is interesting to note that none of these trials reported significant differences between groups, supporting the meta-analysis outcome of no benefit in terms of LICU and LOS with micronutrient supplementation.

The investigator realized that there would be great variation in the type of micronutrients studied, the route of administration, the dosage and the effect of other combination nutrients, thereby making it difficult to attribute any effects to one single or combination of micronutrients. Several pre-planned subgroup analyses were carried out in an attempt to determine which micronutrient strategies were more likely to affect clinical outcomes.

*Single nutrients versus combined micronutrient supplements:* Research on micronutrient supplementation in the critically ill has focused mainly on five micronutrients: selenium, zinc, copper, vitamins C (ascorbic acid) and E ( $\alpha$ -tocopherol). With the exception of one study (that also included vitamin A),<sup>172</sup> all studies included in this review included these micronutrients either singly or in combination. Selenium (as sodium selenite) was the micronutrient of choice in the single nutrient studies (5 of 6). This sub-group analysis was of interest based on the knowledge that interactions between micronutrients are complex and that they function synergistically.<sup>5</sup> Single micronutrient supplementation can counteract the action of other micronutrients and may introduce disturbances in the entire system of overlapping antioxidant defences as antioxidants can turn into pro-oxidants if auxiliary systems for radical scavenging are missing.<sup>180</sup> It can thus be hypothesized that combined micronutrient strategies will have better treatment effects and clinical outcomes. The Heyland et al.<sup>130</sup> systematic review found evidence to the contrary by determining that single nutrients were associated with a significant reduction in mortality ( $p=0.04$ ), and combined micronutrients were not ( $p=0.67$ ). This updated systematic review found that single nutrients (selenium and zinc) were associated with a trend towards lower mortality (borderline statistical significance at  $p=0.06$ ). On the other hand combined micronutrients were not associated with a significant

reduction in mortality. Interestingly, a sensitivity analysis (again excluding Galley 1997<sup>167</sup>) revealed that combined micronutrient supplementation was now associated with a significant reduction in overall mortality. The Galley 1997 trial<sup>167</sup> was excluded with good reason – the other studies had intervention durations ranging from 5-28 days (with an average of  $11.2 \pm 6.4$  days), with Galley 1997 only providing intervention (in the form of  $\alpha$ -tocopherol and ascorbic acid) for 6 hours. The Cochrane systematic review by Avenell et al.<sup>151</sup> investigating the effects of single nutrient supplementation, specifically selenium supplementation (including the selenium-containing compound ebselen) concluded “there is limited evidence to recommend supplementation of critically ill patients with selenium or ebselen”. They found no statistically significant differences for overall mortality and infectious complications and no clear evidence for the benefits of such supplementation for ventilator days, LICU, LOS or quality of life. They did find that the evidence was more suggestive of a benefit on mortality in the first month for general ICU patients when compared to those with severe pancreatitis ( $p=0.02$ ). The outcome of this systematic review does not indicate a clear benefit for the use of single nutrient supplementation in terms of mortality (although it must be noted that significance was borderline). Bearing in mind that five out of the six single nutrient studies used sodium selenite as intervention, these findings are in line with that of the systematic review by Avenell et al. Although the initial analysis indicated no significant effect for combined supplements on mortality, the sensitivity analysis did indeed indicate a benefit for the use thereof. This finding should at least be considered and supports the prior hypothesis and the rationale underlying the use of combinations of micronutrients, based on observations of the biochemical properties of the endogenous antioxidant network and the fact that micronutrients depend on each other for regeneration in a continued spiral.<sup>2</sup> Neither single nor combined micronutrients had an effect on infectious complications (in line with findings of the Heyland et al.<sup>130</sup> review). When single nutrients were compared to combined micronutrients in terms of effect on LICU and LOS, no findings were significant although it should be noted that aggregating the results of combined micronutrient studies indicated borderline statistical significance in terms of LICU ( $p=0.05$ ).

*Route of delivery:* The majority of trials delivered micronutrients intravenously (11 of 15), with two using purely the enteral route and a further two using a combined

enteral and intravenous approach. This sub-group analysis was of interest based on the knowledge that the intravenous route is seen as the only reliable method by which micronutrients can be administered in the critically ill.<sup>5</sup> This route guarantees bioavailability in the circulating compartment whilst avoiding absorption problems. Absorption by the enteral route in critically ill patients is unpredictable due to bowel oedema, bowel ischemia, hemodynamic instability, fluid resuscitation and alterations in blood supply.<sup>185</sup> On the other hand, delivering micronutrients to the gut may be beneficial through prevention of the local gut inflammatory response,<sup>186</sup> indicating that both routes, theoretically at least, do have its advantages.<sup>130</sup> When the parenteral route was compared to the enteral route, no findings were significant for mortality, infectious complications, LICU or LOS. Again in a sensitivity analysis (excluding Galley 1997<sup>167</sup>) investigating the effect of parenteral administration on mortality, the overall estimate of treatment effect did approach significance ( $p=0.07$ ). The review by Heyland et al.<sup>130</sup> did find a significant reduction in mortality associated with parenteral administration of antioxidants ( $p=0.02$ ). Interestingly, it has been suggested that failure of some previous randomized trials to demonstrate a treatment effect may be related to the route of delivery of key nutrients.<sup>187</sup> The majority of trials have used the parenteral route for delivery of nutrients and the Heyland et al. review suggest that the larger treatment effects are associated with parenteral administration.<sup>130</sup> However, given the major role of the gastrointestinal tract as a source of cytokine and leukocyte activation and ROS formation, the provision of key nutrients directly to the gastrointestinal tract makes biological sense.<sup>187</sup> It is thus proposed that future studies investigate the use of both parenteral and enteral administration of micronutrients to maximize the opportunity of demonstrating a treatment effect, if one truly exists.

*Study quality:* It is important to recognize that a meta-analysis is only as good as its components, i.e. the trials (and specifically the study quality of the trials) that make up the various outcomes. This systematic review only included randomized controlled trials, as nonrandomized trials (including quasi-randomized trials) tend to show larger (and often “false-positive”) treatment effects than do randomized trials.<sup>11,130</sup> Despite including only randomized trials, the methodological quality of the individual trials ranged from 7 to 13 (out of a possible 14). Although this scoring system has been used in Cochrane reviews,<sup>151</sup> the use of scales and scoring systems are generally

discouraged by the Cochrane Collaboration due to various reported limitations.<sup>11</sup> This group recommends that it is preferable to use simple approaches for assessing validity that can be fully reported. The Collaboration's recommended tool for assessing risk of bias is a domain-based evaluation (Cochrane "risk of bias" tool), and as such this tool was also completed for each of the included studies. Concealment of allocation was confirmed in only seven of the 15 trials, ten trials reported to be blinded and although not always explicitly stated 12 trials undertook intention-to-treat analysis. The trial quality, as reported, was disappointing in the sense that trials often failed to report trial methodology in sufficient detail. The results of the effect of study quality on mortality, infectious complications, LICU and LOS indicated significant findings only for blinded studies and overall mortality and borderline significance for intention-to-treat analysis and overall mortality ( $p=0.06$ ). These findings support the overall findings of possible benefit in terms of mortality.

In an unpublished January 2007 update<sup>188</sup> of the Heyland et al. systematic review found on the internet (two studies added to the original review), the outcome indicates a significant reduction in mortality only which is in line with the findings of this updated systematic review and the previous Heyland et al.<sup>130</sup> review.

## **4.2 SECONDARY OUTCOMES**

*Timing and duration of micronutrient supplementation:* The timing of micronutrient supplementation is important and is probably a key factor as the repletion of micronutrients, and specifically antioxidants, would probably achieve a greater efficacy if given before massive oxidative injury (e.g. severe sepsis or septic shock).<sup>189</sup> The administration of micronutrients was initiated on the first day of study inclusion (the latter usually within 24-72 hours of ICU admission) in all but one study (all studies for the primary objectives), suggesting and supporting the fact that timing of intervention (i.e. during the acute phase of injury) is important. During this acute phase decreased serum levels of micronutrients are seen together with the largest increases in ROS production. Trials in burns have indicated that early intervention determines the antioxidant impact.<sup>163</sup> Despite the fact that no studies could be found comparing early to late micronutrient supplementation in the critically ill, it is reasonable to conclude that micronutrient supplementation should begin early in the course of critical illness to offset the deleterious effects of ROS. Another important

factor to consider is the duration of supplementation, which should be sufficient to produce optimal clinical benefit. It could be argued that the non significant findings (in terms of mortality) of the Galley 1997 trial<sup>167</sup> could be linked to its very short, and arguably insufficient, intervention duration.

*Micronutrients:* The importance of route of administration and single versus combined micronutrient supplements has been discussed under the primary objectives. As stated previously, selenium was the most commonly used single nutrient, whilst vitamins C (ascorbic acid) and E ( $\alpha$ -tocopherol) were the most commonly used micronutrients in studies of combined supplements. Trials in burns have focused mainly on selenium, zinc and copper after low levels of these micronutrients have repeatedly been shown in this patient population and due to the uniqueness of this group in terms of the extensive cutaneous losses of these micronutrients, further contributing to negative micronutrient balances.<sup>190-193</sup> The focus in supplementation trials is clearly on antioxidant nutrients with the aim to modulate the immune and APR by reinforcement of endogenous antioxidant defences.

Of all the antioxidants, selenium has received the most attention in recent supplementation trials. Selenium is involved in many antioxidative as well as immunological and endocrine pathways. It has been described as one of the "cornerstones of antioxidant defenses in acute conditions".<sup>130</sup> In critically ill patients selenium plasma levels are reduced and inversely correlated with mortality.<sup>182</sup> Selenium supplementation has been shown to normalize plasma selenium concentration as well as GPx-3 activity.<sup>182</sup> It has been hypothesized that supplementing with selenium may improve clinical outcomes as selenium is an essential cofactor in glutathione enzymatic function and has favorable effects on cellular immune function.<sup>179</sup> Selenium is also a component of selenoproteins, some of which have important functions such as activation and regulation of thyroid hormones, regeneration of antioxidant systems, reduction in nucleotides in DNA synthesis and cell viability and proliferation.<sup>130,179</sup> It is thus interesting to note that the Cochrane review on selenium in critical illness found weak and insufficient evidence to support routine supplementation of this antioxidant at this stage. The authors do acknowledge that the evidence is weak as a result of the poor methodological

quality of trials, and that further research in the form of large, well-designed, adequately powered trials is necessary.

*Micronutrient doses:* In terms of doses, the strategies employed in the various trials were not directly associated with adverse / deleterious effects, except perhaps for the Siriwardena et al. trial<sup>175</sup> in severe acute pancreatitis that warrants caution. In this trial selenium (1000 µg), ascorbic acid (2 g) and n-acetylcysteine (300 mg/kg) were administered intravenously in decreasing doses for 7 days. It should be noted that in other trials selenium doses of 1000 µg<sup>156</sup> and even recently up to 4000 µg<sup>166</sup> were considered safe. Similarly doses of up to 3 g ascorbic acid have not been associated with deleterious effects.<sup>170</sup> But herein lie the complexity of this heterogenic group and the realization that not all regimes might be applicable to all critically ill patient population groups. Interestingly the authors of the trial administering 4000 µg of selenium hypothesized that a possible explanation for the absence of effect in their study could be an incipient toxicity of sodium selenite counterbalancing the moderate beneficial effect related to selenium infusion.<sup>166</sup> This highlighting again the importance of micronutrient dose-response curves with plateau's that is followed by toxicity if doses are increased.<sup>194</sup> The authors concluded that the dose used in their trial might have been beyond the optimal dose supporting immune defence. Table 4.1 is included in this discussion section to provide an overview and summary of the current state of evidence and to enable comparisons between the doses used in clinical trials and the available recommendations and proposals. This updated systematic review has highlighted the fact that there is still not clarity regarding optimal doses and which patients will benefit most (or will be adversely affected), and that further large multi-centre trials (including dose-finding trials) are necessary. It is of obvious importance to find the optimal dose for micronutrients administration, which is effective without producing a pro-oxidant effect.

**Table 4.1: Recommended doses and doses used in RCTs of selected micronutrients in critical illness**

Micro-nutrient (MN)	RDA for oral feeding (daily) 144,145	UL 195,196	Recommendations for PN			Proposed supplements (in addition to MNs provided by feeding)			Doses in RCTs
			AMA 1979 146,147	Shenkin 1995 148	FDA 2000 149	Berger 2006 <sup>41</sup>		Fuhrman 2002 (/d) <sup>150</sup>	
						Major trauma (5d)	Major burns (14-21d)		
Vitamin C (mg)	60	2000	100	100	200	1000 (IV)	1000 (IV)	500-3000	500-3000 (IV) 133-500 (EN)
Vitamin E (mg)	8 – 10	1000	10	10	10	100 (EN)	100 (EN)	400 (IV) 40 – 1000 (EN)	50-400 (IV) 50-1350 (EN)
Selenium (µg)	55 – 70	400	30 – 60	60	-	300 (IV)	500 (IV)	100 – 400	200-4000 (IV)
Zinc (mg)	12 – 15	40	2.5 – 4	6.5	-	20 (IV)	30 (IV)	10 – 30	12-37.5 (IV)
Copper (mg)	2	10	0.5 – 1.5	1.3	-	-	4 (IV)	-	2.5-3.75 (IV)

AMA: American Medical Association; d: day; EN: Enteral nutrition; FDA: Food and Drug Administration; IV: Intravenous; MN: Micronutrient; PN: Parenteral nutrition; RCT: Randomized controlled trial; RDA: Recommended dietary allowance for healthy people; UL: Tolerable upper intake level (general population)

*Practice issues (administration of micronutrients):* In terms of practice issues it has been reported that micronutrients were administered mainly via the intravenous route (with a few studies using the enteral route alone or in combination with the intravenous route). While the vast majority of studies used continuous IV infusion, some studies have used bolus injections for the first administration of specifically selenium.<sup>156</sup> It is hypothesized that to reduce the binding of NF-κB to DNA with selenite *in vivo*, a bolus administration is perhaps needed to reach high selenite blood concentrations that can not be attained by continuous administration.<sup>194,197</sup> This was cited as one of the possible reasons for the absence of effect in the Forceville 2007 trial<sup>166</sup> of high-dose selenium supplementation in patients with severe septic shock, as apposed to the Angstwurm 2007 trial<sup>156</sup> that demonstrated positive outcomes (and that used bolus injections for initial administration). Experimental studies are required to answer the question of optimal mode of administration.

*Micronutrient status:* The outcome in terms of micronutrient status after supplementation is not surprising and has been described in the literature repeatedly.

Trials indicated plasma levels of circulating micronutrients to be low after trauma or injury (mechanisms described in detail in the literature review), with the majority of trials indicating significant increases in micronutrient concentrations (for some or all of the micronutrients measured) after micronutrient supplementation. This finding is noted and important, but it cannot be assumed that favorable changes to these micronutrient levels will translate into meaningful changes to clinical outcomes, as was shown in many of the trials studied.

*Morbidity:* Various severity of illness scoring systems were utilized in the included studies, mainly to describe patient populations at baseline. Only eight trials reported it as study endpoints, with APACHE II, SAPS II and ISS most often used. Scoring systems are generally constructed by identifying (either by clinical consensus or by statistical analysis) variables that are best related to mortality. Weights are then attributed to those variables to generate a score or a probability using logistic regression. Sensitivity (predicting death) is calculated as the number of correctly predicted non-survivors divided by the total number of deaths, and specificity (predicting survival) is the number of correctly predicted survivors divided by the total number of survivors.<sup>198</sup> These scoring systems thus provide an indication of patient morbidity. The outcomes in terms of morbidity were variable with the majority of trials indicating no difference between groups, although it should be noted that some trials showed a favorable outcome (i.e. significant decreases) in the micronutrient supplemented group. At present these scoring systems seem most valuable for classifying severity of illness and for randomization purposes at baseline. More studies are needed to determine its usefulness as study endpoints and again linking it to meaningful clinical outcomes.

*Course of the APR:* As a further secondary outcome the investigator wanted to assess whether micronutrient supplementation alter the course of the APR as judged by cytokine levels, WBC and/or differential WBC count and other inflammatory markers (specifically CRP). Reporting was found to be highly variable and sparse with only three studies reporting on cytokine levels, four on WBC and/or differential WBC count and seven on CRP. The studies provided information on IL-1, IL-6, IL-8, IL-10 and TNF- $\alpha$  and found no significant differences between groups, with the remaining study showing a significant difference on day 1 after study commencement only. White

blood cells (or leukocytes) are markers of immune responsiveness and the relevant studies provided conflicting outcomes with either benefit to the micronutrient supplemented groups or no benefit when compared between groups. Although most studies reported decreasing CRP levels, only one of the seven studies found a significant difference between groups at one point in time. The variability and scarcity of data make it impossible to reach firm conclusions regarding the effect of micronutrient supplementation on course of the APR, and again the need for further studies are highlighted.

*Oxidative stress:* The importance of oxidative stress in the critical care setting is well-known and is discussed in the literature review. Oxidative stress defines an imbalance in production of oxidizing chemical species and their effective removal by protective antioxidants and scavenger enzymes.<sup>199</sup> Oxidative stress can aggravate organ injury and thus overall clinical outcome; therefore the interest in micronutrient and specifically antioxidant supplementation trials to try and counteract this imbalance. Several methods have been developed to monitor in vivo oxidative stress: direct quantification of reactive species by electron spin resonance and indirect methods such as determination of antioxidants and total antioxidant capacity and detection of oxidized biological markers, the "biomarkers" of oxidative stress, including products of lipidperoxidation (malondialdehyde, 4-hydroxynonenal, isoprostanes, oxidized-LDL), protein oxidation (hydroxyl and carbonyls), and measurements of DNA damage (high-performance liquid chromatography, gas chromatography).<sup>199</sup> Two-thirds of trials included in this systematic review used an array of plasma variables as markers of oxidative stress. The oxidative stress signals observed in the included trials and their corresponding plasma variables included increased lipid peroxidation [MDA (TBAR) and F2-isoprostanes], increased NO synthesis (nitrates) and decreased circulating antioxidants (GSPHx, GSH, TAC, ascorbic acid,  $\alpha$ -tocopherol, selenium and zinc). For GSHPx (selenoenzyme glutathione peroxidase), results were mostly in favor of the micronutrient supplemented groups, with significant increases in and between groups reported. These positive findings support the potential for intravenous sodium selenite to improve antioxidant capacity in participants. The GSH/GSSG ratio was only reported in one study with no difference between groups. MDA (TBARS) and F2-isoprostane results were highly variable (ranging from significant decreases in experimental groups to no difference between groups). Interestingly, for

TAC one study reported an early unexpected significant decrease in the experimental group, whilst the other study reported TAC unaffected by supplementation. The authors of the study that indicated a significant decrease hypothesized that the unexpected early fall in plasma TAC with supplementation may reflect mobilization of antioxidant defenses.<sup>159</sup> The one study to report on nitrates indicated a significant increase in the experimental group.<sup>156</sup> As nitrite production occurs from both NO in the absence of superoxide and also from peroxynitrite, the increased nitrite concentrations subsequent to antioxidant administration may therefore reflect a further increase in NO generation in the patients receiving antioxidants. Again variable results make it difficult to reach firm conclusions, although several studies did show alterations of single parameters associated with oxidative stress or antioxidant potential during the course of illness. However, up to now, no single parameter can be recommended as the gold standard to define the redox status of patients.<sup>199</sup> Therefore, a cluster of different methodologies (oxidized biological molecules and consumption of antioxidants) could be a better way to assess in vivo oxidative stress.

#### **4.3 STUDY LIMITATIONS**

Search results in this study were limited to English language studies. This was identified to be a limiting factor of this review as imposing language restrictions can introduce bias and influence the precision of the systematic review.<sup>11</sup> Furthermore, searching excluded hand-searching and other exhaustive measures that would typically form part of the gold standard i.e. a Cochrane systematic review.<sup>11</sup> It must however be noted that a great many supposedly good quality systematic reviews published in reputable journals do not employ the full strategies as proposed by the Cochrane Collaboration. These limitations were however thought to be acceptable within the scope of a Masters project with definite limitations in terms of manpower and funding. In addition, the general limitations of systematic reviews and meta-analyses (as discussed in section 1.2.2) should always be borne in mind when interpreting results.

## **CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS**

This updated systematic review and meta-analysis indicates that micronutrient supplementation in the critically ill may be associated with a reduction in overall mortality, and specifically 28 day mortality. However, the results for overall mortality should be interpreted with caution as publication or other sources of bias could, to some extent, account for some of the apparent benefit observed. In terms of mortality the findings of this review therefore support and strengthen the findings of the Heyland et al. review.<sup>130</sup> Results of the study quality sub-group analysis further strengthen to some extent the mortality findings. With the exception of one trial that warrants caution, micronutrient supplementation was not associated with adverse / deleterious effects. Nutrition support practitioners should however always bear in mind that “more is not necessarily better” and that micronutrients have dose-response curves with toxicity at high levels of intake. Furthermore in this group there exists the potential for creating a pro-oxidant microenvironment with deleterious patient outcomes that should always be borne in mind.

This review found no significant effect for micronutrient supplementation on infectious complications, further supporting and strengthening the Heyland et al.<sup>130</sup> findings and the fact that the observed mortality effect was probably mediated by other mechanisms (e.g. improved organ function). This review was the first to report on LICU and LOS and found that these outcomes were unaffected by micronutrient supplementation.

In contrast to the Heyland et al. review but in line with the Avenell et al. selenium review, this review did not find a clear significant reduction in overall mortality associated with single nutrients (although results were borderline statistically significant and thus probably indicating a trend toward lower mortality). Interestingly the sensitivity analysis indicated that combined micronutrient supplements were associated with a reduction in mortality, thus supporting the hypothesis that combined strategies might have a better treatment effect and clinical outcome based on the synergistic functioning of micronutrients. Further supporting this hypothesis is the finding that combined micronutrient supplementation indicated borderline statistical significance in terms of LICU.

In contrast to the Heyland et al. review this review did not find that parenteral administration is superior to enteral administration in terms of effect on mortality, infectious complications, LICU and LOS. Again a sensitivity analysis of the effect of parenteral administration on mortality did approach significance. It must be noted though that only a limited number of trials (n=2) made use of the enteral route alone, therefore making comparisons difficult and the inferences weak.

Timing and duration of micronutrient administration are probably key factors to ensure optimal clinical benefit, with early administration and sufficient duration (yet to be defined) suggested. The focus in supplementation trials is clearly on antioxidant nutrients with the aim to modulate the immune and APR by reinforcement of endogenous antioxidant defences. This updated systematic review has highlighted the fact that there is still not clarity regarding optimal doses and which patients will benefit most (or will be adversely affected), and that further large multi-centre trials (including dose-finding trials) are necessary. Experimental studies are required to answer the question of optimal mode of administration (e.g. continuous infusion versus bolus administration).

Micronutrient status after injury is characterized by low plasma levels with subsequent increases after supplementation. It cannot however be assumed that favorable changes to micronutrient levels will translate into meaningful changes to clinical outcomes, and warrants further investigation. At present various scoring systems are used in clinical trials (as indicators of patient morbidity) and seem most valuable for classifying severity of illness and for randomization purposes at baseline. More studies are needed to determine its usefulness as study endpoints and again linking it to meaningful clinical outcomes. The variability and scarcity of data make it extremely difficult to reach firm conclusions regarding the effect of micronutrient supplementation on course of the APR, and again the need for further studies are highlighted. Several studies indicated alterations of single parameters associated with oxidative stress or antioxidant potential during the course of illness. The oxidative stress signals and corresponding plasma variables observed in the trials included increased lipid peroxidation [MDA (TBAR) and F2-isoprostanes], increased NO synthesis (nitrates) and decreased circulating antioxidants (GSPHx, GSH, TAC, ascorbic acid,  $\alpha$ -tocopherol, selenium and zinc). At present no single parameter can

be recommended as the gold standard to define the redox status of patients and therefore, a group of different methodologies could be a better way to assess oxidative stress in vivo.

This systematic review provides an update and includes the latest micronutrient supplementation trials in the critically ill. Despite the emergence of more trials as well as larger, multi-centre trials, the definitive answers remain elusive in this complex heterogenic group of patients. This review does support potential benefit in terms of some clinical outcomes, but also highlights that caution is warranted in terms of micronutrients administered, doses and specific patient populations targeted as nutrient interactions and risk of toxicity are not clearly defined in critical illness. Once more the conclusion is that there is a need for more large multi-centre prospective randomized controlled trials to assess the effects of different types and doses of micronutrient supplementation in selected groups of patients with different types of critical illness. It has been suggested that targeting the appropriate patient populations most likely to benefit (i.e. the more severely ill patients), considering the route of delivery (possibly a combination of parenteral and enteral) as well as attention to doses administered might maximize the opportunity of demonstrating a treatment effect, if one truly exist. These are all strategies to consider in future trials. Furthermore, no trials have investigated costs or economic outcomes, important aspects that should be assessed in future trials. In practical terms it is clear that micronutrients should be provided at, at least, the current available recommended doses to prevent overt clinical deficiencies. For other claims/indications and higher doses this systematic review and the literature consistently indicate that the risk (adverse effects) to benefit (mortality) ratio may be favourable, and if such higher doses are used in practice it should be within the dose range that the current experience covers and for the clinical settings studied only.

## **Further Recommendations**

### ***Ongoing trials***

The investigator is aware of at least two very large multi-centre randomized controlled trials (the first two of this size in this area of research)<sup>187,200</sup> currently underway that should shed further light and hopefully provide further answers to better define the requirement of micronutrients in the critically ill. These two trials will

be essential additions to this updated systematic review once the results are available.

The REDOXS<sup>®</sup> (REducing Deaths due to OXidative Stress) study<sup>187</sup> is a multi-centre study aiming to recruit 1200 mechanically-ventilated critically ill patients. Patients are randomized into four groups, i.e. glutamine, antioxidant, glutamine and antioxidant or placebo group. The experimental groups will receive glutamine (enterally and parenterally) and/or an antioxidant cocktail [i.e. receive 500 µg Se parenterally and the following vitamins and minerals administered enterally (mg): Se, 300 µg; Zn, 20; β-carotene, 10; vitamin E, 500; vitamin C, 1500]. Intervention to start within 24 hours of ICU admission and to last for a minimum of 5 days (if transferred out of ICU) and a maximum of 28 days. The first results of this study are expected in 2009.

The SIGNET, Scottish Intensive care Glutamine or seleNium Evaluative Trial,<sup>200</sup> is a multi-centre study aiming to recruit 500 general intensive care patients. Patients are randomized into four groups, i.e. glutamine, selenium, glutamine and selenium or placebo group. The experimental groups will receive glutamine (parenterally) and/or selenium (500 µg parenterally) for seven days. Interventions start the soonest possible and at least within 48 hours of ICU admission. Recruitment is due to finish in August 2008 with results due in 2009.

### ***Foreign language trials***

In order to obtain optimal precision in this systematic review it is advised that foreign language studies should be considered for inclusion in future if possible. Six foreign language studies were identified for possible inclusion but were subsequently excluded due to language constraints (see Appendix 6.8 for bibliographic details). Although all foreign language studies should be individually translated and evaluated, it should be noted that three of these studies have not been classified as randomized controlled trials in the databases that host it and a further study appear to only provide outcomes for the secondary objectives of this review (and thus not the meta-analysis). The remaining two studies<sup>201,202</sup> can be considered potentially eligible studies for the primary outcomes of this systematic review.

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## **Appendices**

**Appendix 6.1: Ethics Approval**

13 November 2006

Mrs J Visser  
Division of Human Nutrition  
Dept of Interdisciplinary Health Sciences

Dear Mrs Visser

**RESEARCH PROJECT : "CURRENT PRACTICES OF MICRONUTRIENT  
SUPPLEMENTATION IN THE CRITICALLY ILL"  
PROJECT NUMBER : 99/035**

The e-mail correspondence regarding the change of the title your research project refers.

The changed protocol was scrutinised and I wish to confirm that the Chairman of the Committee for Human Research approved the change of the project title from "The micronutrient status and requirements of patients undergoing gastro-intestinal surgery" to "Current practices of micronutrient supplementation in the critically ill".

This approval will be submitted to the Committee for ratification.

Yours faithfully.

**CJ VAN TONDER**  
**NAVORSINGSONTWIKKELING EN -STEUN (TYGERBERG)**  
Tel: +27 21 938 9207 / E-pos: cjvt@sun.ac.za

CJVT/pm

**Appendix 6.2: Phase 2: Selection of studies - Study Eligibility (Filled lines denote included studies; Studies included over 2 pages; Y: Yes; N: No)**

Study ID	Study inclusion criteria: Patients					Study inclusion criteria (continued): Outcomes									
	A tick in EVERY column is necessary for study INCLUSION					A tick in AT LEAST 1 column (or more) is necessary for study INCLUSION									
	Adults	Men & non-pregnant, non-lactating women	Critically ill patients	Micronutrient supplementation	English language	Micronutrient status	Cytokine levels	WBC/diff WBC count	Inflammatory markers	Morbidity	Mortality	Sepsis (level: oxidative stress)	Length: hospital stay	Length: ICU stay	Infectious complications
S1					Y										
S2	Y	Y	Y	Y	Y				Y			Y			
S3	Y	Y	Y	Y	Y	Y			Y	Y	Y	Y		Y	Y
S4					Y										
S5	Y	Y	Y	Y	Y	Y				Y					
S6	Y	Y	Y	Y	Y	Y				Y	Y				
S7	Y	Y	Y	N	Y										
S8					Y										
S9					Y										
S10	Y	Y	Y	Y	Y						Y	Y	Y	Y	
S11	Y	Y	N	Y	Y	Y						Y	Y	Y	
S12	Y	Y	Y	Y	Y	Y									
S13					N										
S14	Y	Y	Y	Y	Y	Y		Y	Y		Y	Y	Y	Y	Y
S15	Y	Y	Y	Y	Y	Y	Y	Y	Y		Y	Y	Y	Y	Y
S16					Y										
S17					Y										
S18	Y	Y	Y	Y	Y	Y									
S19	Y	Y	Y	Y	Y	Y			Y						
S20	Y	Y	Y	Y	Y	Y		Y	Y				Y		
S21	Y	Y	Y	Y	Y	Y			Y			Y			
S22					Y										
S23					N										
S24	Y	Y	Y	Y	Y	Y					Y	Y		Y	Y
S25					Y										
S26	Y	Y	Y	Y	Y	Y					Y		Y	Y	Y
S27	Y	Y	Y	N	Y	Y			Y					Y	
S28					Y										
S29					Y										
S30					Y										

Study ID	Study exclusion criteria								Included or Excluded
	A tick in ANY column indicates study EXCLUSION								
	No outcomes considered in the objectives	Multiple nutrients	Foreign language	Review articles	Abstracts Letters Editorials Comments	No results available	Animal studies	Lack of randomization	
S1						Y			EXCLUDED
S2		Y							EXCLUDED
S3									INCLUDED
S4				Y					EXCLUDED
S5									INCLUDED
S6									INCLUDED
S7	Y								EXCLUDED
S8				Y					EXCLUDED
S9					A				EXCLUDED
S10									EXCLUDED
S11									EXCLUDED
S12		Y							EXCLUDED
S13			Y	Y					EXCLUDED
S14									INCLUDED
S15									INCLUDED
S16					A				EXCLUDED
S17					A				EXCLUDED
S18									INCLUDED
S19								Y	EXCLUDED
S20								Y	EXCLUDED
S21								Y	EXCLUDED
S22				Y					EXCLUDED
S23			Y	Y					EXCLUDED
S24									EXCLUDED
S25					A				EXCLUDED
S26									INCLUDED
S27									EXCLUDED
S28					EC				EXCLUDED
S29				Y					EXCLUDED
S30				Y					EXCLUDED

Study ID	Study inclusion criteria: Patients					Study inclusion criteria (continued): Outcomes									
	A tick in EVERY column is necessary for study INCLUSION					A tick in AT LEAST 1 column (or more) is necessary for study INCLUSION									
	Adults	Men & non-pregnant, non-lactating women	Critically ill patients	Micronutrient supplementation	English language	Micronutrient status	Cytokine levels	WBC/diff WBC count	Inflammatory markers	Morbidity	Mortality	Sepsis (level: oxidative stress)	Length: hospital stay	Length: ICU stay	Infectious complications
S31					Y										
S32					Y										
S33	Y	Y	Y	Y	Y	Y		Y					Y		Y
S34					Y										
S35					Y										
S36					Y										
S37					N										
S38					N										
S39					N										
S40	Y	Y	Y	Y	Y					Y					
S41					Y										
S42					Y										
S43					Y										
S44	Y	Y	Y	N	Y	Y									
S45	Y	Y	N	Y	Y	Y	Y		Y						Y
S46	Y	Y	Y	Y	Y				Y						Y
S47					Y										
S48					Y										
S49					Y										
S50	Y	Y	Y	Y	Y					Y		Y	Y	Y	Y
S51					Y										
S52	Y	Y	Y	Y	Y	Y		Y	Y	Y					
S53					Y										
S54	Y	Y	Y	Y	Y					Y	Y	Y			Y
S55					Y										
S56					Y										
S57					Y										
S58	Y	Y	Y	N	Y					Y					
S59					Y										
S60					Y										

Study ID	Study exclusion criteria								Included or Excluded
	A tick in ANY column indicates study EXCLUSION								
	No outcomes considered in the objectives	Multiple nutrients	Foreign language	Review articles	Abstracts Letters Editorials Comments	No results available	Animal studies	Lack of randomization	
S31				Y					EXCLUDED
S32					A				EXCLUDED
S33									INCLUDED
S34					L				EXCLUDED
S35				Y					EXCLUDED
S36				Y					EXCLUDED
S37			Y	Y					EXCLUDED
S38			Y	Y					EXCLUDED
S39			Y	Y					EXCLUDED
S40		Y							EXCLUDED
S41				Y					EXCLUDED
S42				Y					EXCLUDED
S43				Y					EXCLUDED
S44									EXCLUDED
S45									EXCLUDED
S46		Y							EXCLUDED
S47				Y					EXCLUDED
S48				Y					EXCLUDED
S49				Y					EXCLUDED
S50		Y							EXCLUDED
S51				Y					EXCLUDED
S52									INCLUDED
S53				Y					EXCLUDED
S54									INCLUDED
S55				Y					EXCLUDED
S56				Y					EXCLUDED
S57					EC				EXCLUDED
S58									EXCLUDED
S59				Y					EXCLUDED
S60				Y					EXCLUDED

Study ID	Study inclusion criteria: Patients					Study inclusion criteria (continued): Outcomes									
	A tick in EVERY column is necessary for study INCLUSION					A tick in AT LEAST 1 column (or more) is necessary for study INCLUSION									
	Adults	Men & non-pregnant, non-lactating women	Critically ill patients	Micronutrient supplementation	English language	Micronutrient status	Cytokine levels	WBC/diff WBC count	Inflammatory markers	Morbidity	Mortality	Sepsis (level: oxidative stress)	Length: hospital stay	Length: ICU stay	Infectious complications
S61					Y										
S62	Y	Y	N	Y	Y	Y					Y		Y		
S63					Y										
S64	Y	Y	Y	Y	Y	Y									
S65	Y	Y	Y	Y	Y						Y		Y	Y	Y
S66	Y	Y	Y	N	Y	Y					Y	Y		Y	Y
S67	Y	Y	Y	Y	Y									Y	Y
S68	Y	Y	Y	Y	Y	Y					Y	Y			
S69					Y										
S70					N										
S71					Y										
S72	Y	Y	N	Y	Y			Y			Y				
S73					Y										
S74					Y										
S75					Y										
S76					Y										
S77					Y										
S78	Y	Y	Y	Y	Y	Y					Y	Y		Y	
S79					Y										
S80	Y	Y	Y	Y	Y	Y					Y		Y	Y	Y
S81					Y										
S82					N										
S83					N										
S84					N										
S85					Y										
S86					N										
S87	Y	Y	Y	Y	Y	Y						Y			
S88	Y	Y	N	Y	Y	Y			Y						
S89					Y										
S90	Y	Y	Y	Y	Y	Y									

Study ID	Study exclusion criteria								Included or Excluded
	A tick in ANY column indicates study EXCLUSION								
	No outcomes considered in the objectives	Multiple nutrients	Foreign language	Review articles	Abstracts Letters Editorials Comments	No results available	Animal studies	Lack of randomization	
S61				Y					EXCLUDED
S62									EXCLUDED
S63				Y					EXCLUDED
S64									EXCLUDED
S65									INCLUDED
S66									EXCLUDED
S67		Y							EXCLUDED
S68									INCLUDED
S69					A				EXCLUDED
S70			Y						EXCLUDED
S71				Y					EXCLUDED
S72		Y							EXCLUDED
S73					A				EXCLUDED
S74				Y					EXCLUDED
S75				Y					EXCLUDED
S76				Y					EXCLUDED
S77				Y					EXCLUDED
S78		Y							EXCLUDED
S79					C				EXCLUDED
S80		Y							EXCLUDED
S81				Y					EXCLUDED
S82			Y						EXCLUDED
S83			Y						EXCLUDED
S84			Y						EXCLUDED
S85				Y					EXCLUDED
S86			Y						EXCLUDED
S87								Y	EXCLUDED
S88									EXCLUDED
S89				Y					EXCLUDED
S90								Y	EXCLUDED

Study ID	Study inclusion criteria: Patients					Study inclusion criteria (continued): Outcomes									
	A tick in EVERY column is necessary for study INCLUSION					A tick in AT LEAST 1 column (or more) is necessary for study INCLUSION									
	Adults	Men & non-pregnant, non-lactating women	Critically ill patients	Micronutrient supplementation	English language	Micronutrient status	Cytokine levels	WBC/diff WBC count	Inflammatory markers	Morbidity	Mortality	Sepsis (level: oxidative stress)	Length: hospital stay	Length: ICU stay	Infectious complications
S91					Y										
S92	Y	Y	Y	Y	Y	Y		Y							Y
S93					N										
S94	Y	Y	Y	N	Y										
S95					Y										
S96	Y	Y	Y	N	Y	Y									
S97					Y										
S98	Y	Y	Y	Y	Y	Y		Y			Y		Y	Y	Y
S99					Y										
S100					Y										
S101	Y	Y	Y	Y	Y	Y					Y				
S102					Y										
S103	Y	Y	Y	Y	Y	Y			Y		Y			Y	Y
S104	Y	Y	Y	N	Y	Y					Y				
S105					Y										
S106	Y	Y	N	N	Y										
S107	Y	Y	Y	Y	Y	Y				Y	Y			Y	Y
S108	Y	Y	Y	Y	Y	Y									
S109					N										
S110					Y										
S111					Y										
S112	Y	Y	Y	Y	Y	Y									
S113					Y										
S114	Y	Y	Y	Y	Y						Y				
S115	Y	Y	Y	Y	Y						Y	Y	Y	Y	Y
S116					Y										
S117					Y										
S118	Y	Y	Y	Y	Y	Y					Y	Y		Y	Y
S119					Y										
S120					Y										

Study ID	Study exclusion criteria								Included or Excluded
	A tick in ANY column indicates study EXCLUSION								
	No outcomes considered in the objectives	Multiple nutrients	Foreign language	Review articles	Abstracts Letters Editorials Comments	No results available	Animal studies	Lack of randomization	
S91				Y					EXCLUDED
S92									INCLUDED
S93			Y	Y					EXCLUDED
S94	Y								EXCLUDED
S95							Y		EXCLUDED
S96									EXCLUDED
S97				Y					EXCLUDED
S98		Y							EXCLUDED
S99				Y					EXCLUDED
S100				Y					EXCLUDED
S101								Y	EXCLUDED
S102					A				EXCLUDED
S103									INCLUDED
S104									EXCLUDED
S105				Y					EXCLUDED
S106	Y								EXCLUDED
S107									INCLUDED
S108		Y							EXCLUDED
S109			Y	Y					EXCLUDED
S110					EC				EXCLUDED
S111				Y					EXCLUDED
S112		Y							EXCLUDED
S113				Y					EXCLUDED
S114		Y							EXCLUDED
S115									INCLUDED
S116				Y					EXCLUDED
S117					A				EXCLUDED
S118									INCLUDED
S119				Y					EXCLUDED
S120				Y					EXCLUDED

Study ID	Study inclusion criteria: Patients					Study inclusion criteria (continued): Outcomes									
	A tick in EVERY column is necessary for study INCLUSION					A tick in AT LEAST 1 column (or more) is necessary for study INCLUSION									
	Adults	Men & non-pregnant, non-lactating women	Critically ill patients	Micronutrient supplementation	English language	Micronutrient status	Cytokine levels	WBC/diff WBC count	Inflammatory markers	Morbidity	Mortality	Sepsis (level: oxidative stress)	Length: hospital stay	Length: ICU stay	Infectious complications
S121	Y	Y	Y	N	Y	Y									
S122	Y	Y	Y	N	Y										
S123	Y	Y	N	Y	Y										
S124	Y	Y	Y	Y	Y	Y									
S125					Y										
S126					Y										
S127					Y										
S128	Y	Y	Y	Y	Y	Y									
S129					Y										
S130					Y										
S131	Y	Y	Y	Y	Y	Y									
S132					Y										
S133					Y										
S134	Y	Y	Y	Y	Y					Y				Y	
S135	Y	Y	Y	Y	Y	Y			Y	Y	Y	Y	Y	Y	
S136					Y										
S137					Y										
S138					Y										
S139	Y	Y	Y	Y	Y	Y				Y	Y	Y			Y
S140	Y	Y	Y	Y	Y										
S141	Y	Y	N	Y	Y	Y			Y		Y				
S142	Y	Y	N	Y	Y	Y			Y						
S143	Y	Y	Y	Y	Y	Y				Y				Y	
S144	Y	Y	N	Y	Y										
S145					Y										
S146	Y	Y	Y	Y	Y	Y				Y					
S147					Y										
S148					Y										
S149					N										
S150					Y										
S151					N										

Study ID	Study exclusion criteria								Included or Excluded
	A tick in ANY column indicates study EXCLUSION								
	No outcomes considered in the objectives	Multiple nutrients	Foreign language	Review articles	Abstracts Letters Editorials Comments	No results available	Animal studies	Lack of randomization	
S121									EXCLUDED
S122	Y								EXCLUDED
S123	Y								EXCLUDED
S124									INCLUDED
S125				Y					EXCLUDED
S126				Y					EXCLUDED
S127					A				EXCLUDED
S128									INCLUDED
S129					C				EXCLUDED
S130					L				EXCLUDED
S131								Y	EXCLUDED
S132				Y					EXCLUDED
S133				Y					EXCLUDED
S134		Y							EXCLUDED
S135									INCLUDED
S136				Y					EXCLUDED
S137				Y					EXCLUDED
S138				Y					EXCLUDED
S139								Y	EXCLUDED
S140	Y	Y							EXCLUDED
S141									EXCLUDED
S142									EXCLUDED
S143		Y							EXCLUDED
S144	Y								EXCLUDED
S145				Y					EXCLUDED
S146									INCLUDED
S147				Y					EXCLUDED
S148							Y	Y	EXCLUDED
S149			Y	Y					EXCLUDED
S150				Y					EXCLUDED
S151			Y						EXCLUDED

**Appendix 6.3: Characteristics of excluded studies: reasons for study exclusion**

<b>STUDY ID</b>	<b>STUDY AND YEAR</b>	<b>REASON FOR EXCLUSION</b>
S1	Andrews 2007	Study protocol for trial (results expected 2009)
S2	Angdin 2003	Multiple nutrients; Pre-op supplementation in elective surgery patients
S4	Angstwurm 2006	Review
S7	Askari 1982	No micronutrient supplementation
S8	Avenell 2004	Systematic review
S9	Baines 1996	Abstract
S10	Barquist 1998	Multiple and different treatment modalities; Lack of randomization (historical controls)
S11	Bartels 2004	Pre-op supplementation in elective surgery patients
S12	Beale 2008	Multiple nutrients
S13	Berger 1998	Foreign language; Review
S16	Berger 2002	Abstract
S17	Berger 2004	Abstract
S19	Berger 1996	Lack of randomization
S21	Berger 1995	Lack of randomization (historical controls)
S20	Berger 1994	Lack of randomization; Quasi-randomized
S22	Berger 2007	Review
S23	Berger 2001	Foreign language; Review
S24	Berger 2006	Aggregation of 2 RCT's already included (S33 and S15/S18)
S25	Berger 2005	Abstract
S27	Berger 2004	No micronutrient supplementation
S28	Berger 2007	Editorial comment
S29	Berger 2007	Review
S30	Berger 2006	Review
S31	Berger 2006	Review
S32	Berger 2005	Abstract
S34	Berger 2006	Letter to the Editor
S35	Berger 2006	Review
S36	Berger 2005	Review
S37	Berger 2006	Foreign language; Review
S38	Berger 1995	Foreign language; Review
S39	Berger 1995	Foreign language; Review
S40	Bertolini 2003	Multiple nutrients
S41	Biesalski 2007	Review
S42	Bistrrian 2006	Review
S43	Bongers 2006	Review
S44	Bradley 1978	No micronutrient supplementation
S45	Braunschweig 1997	Not critically ill
S46	Brown 1994	Multiple nutrients
S47	Bulger 2003	Review
S48	Bulger 2001	Review
S49	Calder 2007	Review
S50	Caparrós 2001	Multiple nutrients
S51	Cartwright 2004	Review
S53	Coleman 2001	Review
S55	Crimi 2006	Review
S56	Crimi 2006	Review
S57	Cross 2006	Editorial comment
S58	Cruickshank 1988	No micronutrient supplementation
S59	Davies 2007	Review

STUDY ID	STUDY AND YEAR	REASON FOR EXCLUSION
S60	Deitch 1995	Review
S61	Dhaliwal 2005	Review
S62	Du 2003	Not critically ill
S63	Eaton 2006	Review
S64	Faure 1991	Pre-op supplementation in elective surgery patients
S66	Forceville 1998	No micronutrient supplementation
S67	Gadek 1999	Multiple nutrients
S69	Garcia Garmendia 1998	Abstract
S70	Gärtner 1999	Foreign language
S71	Geoghegan 2006	Review
S72	Gogos 1998	Not critically ill; Multiple nutrients
S73	Grau 2001	Abstract
S74	Grimble 1997	Review
S75	Grimble 1998	Review
S76	Heyland 2006	Review
S77	Heyland 2005	Systematic review
S78	Heyland 2007	Multiple nutrients
S79	Heyland 2007	Comment on S65
S80	Kieft 2005	Multiple nutrients
S81	Kreymann 2006	Review; Practice guidelines
S82	Kuklinski 1992	Foreign language
S83	Kuklinski 1991	Foreign language
S84	Kuklinski 1995	Foreign language
S85	Lafrance 2005	Review
S86	Lehmann 1997	Foreign language
S87	Lehmann 1998	Lack of randomization
S88	Leichtle 2006	Not critically ill
S89	Levy 2002	Review
S90	Long 2003	Lack of randomization
S91	Lovat 2003	Review
S93	Manzanares Castro 2007	Foreign language; Review
S94	Mashour 2000	No micronutrient supplementation
S95	Matsuda 1992	Animal trial
S96	McConachie 1988	No micronutrient supplementation
S97	Mechanick 2002	Review
S98	Mendez 1997	Multiple nutrients
S99	Meydani 1998	Review
S100	Meyer 1994	Review
S101	Mingjian 1992	Lack of randomization
S102	Mishra 2005	Abstract
S104	Mishra 2005	No micronutrient supplementation
S105	Mishra 2007	Review
S106	Murray 1978	Not critically ill; No micronutrient supplementation
S108	Nelson 2003	Multiple nutrients
S109	Nitenberg 2003	Foreign language; Review
S110	Ochoa 2008	Editorial comment
S111	Oldham 1998	Review
S112	Pacht 2003	Multiple nutrients
S113	Pingleton 2001	Review
S114	Pontes-Arruda 2006	Multiple nutrients
S116	Powell-Tuck 2007	Review
S117	Preiser 1998	Abstract
S119	Prelack 2007	Review

<b>STUDY ID</b>	<b>STUDY AND YEAR</b>	<b>REASON FOR EXCLUSION</b>
S120	Prelack 2001	Review
S121	Quasim 2005	No micronutrient supplementation
S122	Rabe 2002	No micronutrient supplementation; No outcomes considered in the objectives
S123	Rabl 1995	Not critically ill; No outcomes considered in the objectives
S125	Roth 2004	Review
S126	Rotstein 2001	Review
S127	Rümelin 2001	Abstract
S129	Sacks 2003	Comment
S130	Schomburg 2007	Letter to the Editor
S131	Seeger 1987	Lack of randomization; Healthy volunteers
S132	Shenkin 1997	Review
S133	Shenkin 2006	Review
S134	Singer 2006	Multiple nutrients
S136	Slone 2004	Review
S137	Soeters 2001	Review
S138	Stawicki 2007	Review
S139	Tanaka 2000	Lack of randomization; Quasi-randomized
S140	Theilla 2007	Multiple nutrients; No outcomes considered in the objectives
S141	Ullegaddi 2006	Not critically ill
S142	Ullegaddi 2004	Not critically ill
S143	Van Iperen 2000	Multiple nutrients
S144	Vaxman 1996	Not critically ill; No outcomes considered in the objectives
S145	Wheatley 2006	Review
S147	Zanello 2006	Review
S148	Zang 2007	Animal trial
S149	Zazzo 2002	Foreign language; Review
S150	Ziegler 1997	Review
S151	Zimmermann 1997	Foreign language

**Appendix 6.4: Bibliographic information and study identification numbers of the excluded studies**

Study ID	Study reference (excluded)
S1	Andrews PJ, Avenell A, Noble DW, Campbell MK, Battison CG, Croal BL, et al. for The Trials Management Group. Randomised trial of glutamine and selenium supplemented parenteral nutrition for critically ill patients. Protocol Version 9, 19 February 2007 known as SIGNET (Scottish Intensive care Glutamine or seleNium Evaluative Trial). <i>Trials</i> 2007; 8(1): 25-39.
S2	Angdin M, Settergren G, Starkopf J, Zilmer M, Zilmer K, Vaage J. Protective effect of antioxidants on pulmonary endothelial function after cardiopulmonary bypass. <i>J Cardiothorac Vasc Anesth</i> 2003; 17(3): 314-320.
S4	Angstwurm MW, Gaertner R. Practicalities of selenium supplementation in critically ill patients. <i>Curr Opin Clin Nutr Metab Care</i> 2006; 9(3): 233-238.
S7	Askari A, Long CL, Blakemore WS. Net metabolic changes of zinc, copper, nitrogen, and potassium balances in skeletal trauma patients. <i>Metabolism</i> 1982; 31(12): 1185-1193.
S8	Avenell A, Noble DW, Barr J, Engelhardt T. Selenium supplementation for critically ill adults. <i>Cochrane Database Syst Rev</i> 2004; 18;(4): CD003703.
S9	Baines M, Wardle CA, Berger MM, Pannatier A, Chioléro R, Shenkin A. Markers of oxidative protection in major trauma patients following trace element supplementation. <i>Clin Nutr</i> 1996; 15(Suppl 1): 43.
S10	Barquist E, Kirton O, Windsor J, Hudson-Civetta J, Lynn M, Herman M, et al. The impact of antioxidant and splanchnic-directed therapy on persistent uncorrected gastric mucosal pH in the critically injured trauma patient. <i>J Trauma</i> 1998; 44(2): 355-360.
S11	Bartels M, Biesalski HK, Engelhart K, Sendlhofer G, Rehak P, Nagel E. Pilot study on the effect of parenteral vitamin E on ischemia and reperfusion induced liver injury: a double blind, randomized, placebo-controlled trial. <i>Clin Nutr</i> 2004; 23(6): 1360-1370.
S12	Beale RJ, Sherry T, Lei K, Campbell-Stephen L, McCook J, Smith J, et al. Early enteral supplementation with key pharmaconutrients improves sequential organ failure assessment score in critically ill patients with sepsis: outcome of a randomized, controlled, double-blind trial. <i>Crit Care Med</i> 2008; 36(1): 131-144.
S13	Berger M. Nutrition de l'agressé: quelle est la place des micronutriments? [Nutrition of the stressed patient: which place for the micronutrients?] <i>Nutr Clin Métabol</i> 1998; 12(Suppl 1): 197-209.
S16	Berger MM, Baines M, Wardle CA, Cayeux MC, Chioléro R, Shenkin A. Trace element supplements modulate tissue levels, antioxidant status and clinical course after major burns – preliminary results. <i>Clin Nutr</i> 2002; 21 (Suppl): 66.
S17	Berger MM, Binnert C, Baines M, Raffoul W, Cayeux M, Chioléro R, et al. Trace element supplements influence protein metabolism and tissue levels after major burns. <i>Intensive Care Med</i> 2004; 30(Suppl): S61.
S19	Berger MM, Cavadini C, Chioléro R, Dirren H. Copper, selenium, and zinc status and balances after major trauma. <i>J Trauma</i> 1996; 40(1): 103-109.
S20	Berger MM, Cavadini C, Chioléro R, Guinchard S, Krupp S, Dirren H. Influence of large intakes of trace elements on recovery after major burns. <i>Nutrition</i> 1994; 10(4): 327-334.
S21	Berger MM, Chioléro R. Relations between copper, zinc and selenium intakes and malondialdehyde excretion after major burns. <i>Burns</i> 1995; 21(7): 507-512.
S22	Berger MM, Chioléro RL. Antioxidant supplementation in sepsis and systemic inflammatory response syndrome. <i>Crit Care Med</i> 2007; 35(9 Suppl): S584-S590.
S23	Berger MM, Chioléro RL. Apport d'antioxydants en réanimation: pourquoi, lesquels, avec quels objectifs? [Antioxidant supplements in intensive care: why, which, and with what objectives?] <i>Réanimation</i> 2001; 10(6): 527-534.

Study ID	Study reference (excluded)
S24	Berger MM, Eggimann P, Heyland DK, Chioléro RL, Revely JP, Day A, et al. Reduction of nosocomial pneumonia after major burns by trace element supplementation: aggregation of two randomised trials. <i>Crit Care</i> 2006; 10(6): R153.
S25	Berger MM, Eggimann P, Revely JP, Raffoul W, Shenkin A, Chioléro R. Trace element supplements are associated with fewer nosocomial pneumonia after major burns. <i>Intensive Care Med</i> 2005; 31 (Suppl 1): S77.
S27	Berger MM, Shenkin A, Revely JP, Roberts E, Cayeux MC, Baines M, et al. Copper, selenium, zinc, and thiamine balances during continuous venovenous hemodiafiltration in critically ill patients. <i>Am J Clin Nutr</i> 2004; 80(2): 410-416.
S28	Berger MM, Shenkin A. Selenium in intensive care: probably not a magic bullet but an important adjuvant therapy. <i>Crit Care Med</i> 2007; 35(1): 306-307.
S29	Berger MM, Shenkin A. Trace element requirements in critically ill burned patients. <i>J Trace Elem Med Biol</i> 2007; 21(Suppl 1): 44-48.
S30	Berger MM, Shenkin A. Update on clinical micronutrient supplementation studies in the critically ill. <i>Curr Opin Clin Nutr Metab Care</i> 2006; 9(6): 711-716.
S31	Berger MM, Shenkin A. Vitamins and trace elements: practical aspects of supplementation. <i>Nutrition</i> 2006; 22(9): 952-955.
S32	Berger MM, Soguel L, Pinget C, Revely JP, Schindler C, Chioléro RL. Antioxidant supplements modulate clinical course after complex cardiac surgery, and major trauma. <i>Intensive Care Med</i> 2005; 31 (Suppl 1): S32.
S34	Berger MM. Acute copper and zinc deficiency due to exudative losses – substitution versus nutritional requirements. <i>Burns</i> 2006; 32: 393.
S35	Berger MM. Antioxidant micronutrients in major trauma and burns: evidence and practice. <i>Nutr Clin Pract</i> 2006; 21(5): 438-449.
S36	Berger MM. Can oxidative damage be treated nutritionally? <i>Clin Nutr</i> 2005; 24(2): 172-183.
S37	Berger MM. Manipulations nutritionnelles du stress oxydant: état des connaissances [Nutritional manipulation of oxidative stress: review of the evidence]. <i>Nutr Clin Métabol</i> 2006; 20(1): 48-53.
S38	Berger MM. Rôle des oligo-éléments et des vitamines en nutrition périopératoire [Role of trace elements and vitamins in perioperative nutrition]. <i>Ann Fr Anesth Réanim</i> 1995; 14(Suppl 2): 82-94.
S39	Berger MM. Rôle des oligo-éléments et des vitamines en nutrition périopératoire [Role of trace elements and vitamins in perioperative nutrition]. <i>Nutr Clin Métabol</i> 1995; 9(Suppl 1): 91-103.
S40	Bertolini G, Iapichino G, Radrizzani D, Facchini R, Simini B, Bruzzone P, et al. Early enteral immunonutrition in patients with severe sepsis: results of an interim analysis of a randomized multicentre clinical trial. <i>Intensive Care Med</i> 2003; 29(5): 834-840.
S41	Biesalski HK, McGregor GP. Antioxidant therapy in critical care - is the microcirculation the primary target? <i>Crit Care Med</i> 2007; 35(9 Suppl): S577-S583.
S42	Bistran BR, McCowen KC. Nutritional and metabolic support in the adult intensive care unit: key controversies. <i>Crit Care Med</i> 2006; 34(5): 1525-1531.
S43	Bongers T, Griffiths RD. Are there any real differences between enteral feed formulations used in the critically ill? <i>Curr Opin Crit Care</i> 2006; 12(2): 131-135.
S44	Bradley JA, King RF, Schorah CJ, Hill GL. Vitamins in intravenous feeding: a study of water-soluble vitamins and folate in critically ill patients receiving intravenous nutrition. <i>Br J Surg</i> 1978; 65(7): 492-494.
S45	Braunschweig CL, Sowers M, Kovacevich DS, Hill GM, August DA. Parenteral zinc supplementation in adult humans during the acute phase response increases the febrile response. <i>J Nutr</i> 1997; 127: 70-74.
S46	Brown RO, Hunt H, Mowatt-Larsen CA, Wojtysiak SL, Henningfield MF, Kudsk KA. Comparison of specialized and standard enteral formulas in trauma patients. <i>Pharmacotherapy</i> 1994; 14(3): 314-320.

Study ID	Study reference (excluded)
S47	Bulger EM, Maier RV. An argument for vitamin E supplementation in the management of systemic inflammatory response syndrome. <i>Shock</i> 2003; 19: 99-103.
S48	Bulger EM, Maier RV. Antioxidants in critical illness. <i>Arch Surg</i> 2001; 136: 1201-1207.
S49	Calder PC. Immunonutrition in surgical and critically ill patients. <i>Br J Nutr</i> 2007; 98(Suppl 1): S133-S139.
S50	Caparrós T, Lopez J, Grau T. Early enteral nutrition in critically ill patients with a high-protein diet enriched with arginine, fiber, and antioxidants compared with a standard high-protein diet. The effect on nosocomial infections and outcome. <i>J Parenter Enteral Nutr</i> 2001; 25(6): 299-309.
S51	Cartwright MM. The metabolic response to stress: a case of complex nutrition support management. <i>Crit Care Nurs Clin N Am</i> 2004; 16(4): 467-487.
S53	Coleman NA. Antioxidants in critical care medicine. <i>Environ Toxicol Pharmacol</i> 2001; 10(4): 183-188.
S55	Crimi E, Sica V, Slutsky AS, Zhang H, Williams-Ignarro S, Ignarro LJ, et al. Role of oxidative stress in experimental sepsis and multisystem organ dysfunction. <i>Free Radic Res</i> 2006; 40(7): 665-672.
S56	Crimi E, Sica V, Williams-Ignarro S, Zhang H, Slutsky AS, Ignarro LJ, et al. The role of oxidative stress in adult critical care. <i>Free Radic Biol Med</i> 2006; 40(3): 398-406.
S57	Cross CE, Van Asbeck BS, Halliwell B. More antioxidants in sepsis: still paved with uncertainties. <i>Crit Care Med</i> 2006; 34(2): 569-571.
S58	Cruickshank AM, Telfer AB, Shenkin A. Thiamine deficiency in the critically ill. <i>Intensive Care Med</i> 1988; 14(4): 384-387.
S59	Davies AR. Practicalities of nutrition support in the intensive care unit. <i>Curr Opin Clin Nutr Metab Care</i> 2007; 10(3): 284-290.
S60	Deitch EA. Nutritional support of the burn patient. <i>Crit Care Clin</i> 1995; 11(3): 735-750.
S61	Dhaliwal R, Heyland DK. Nutrition and infection in the intensive care unit: what does the evidence show? <i>Curr Opin Crit Care</i> 2005; 11(5): 461-467.
S62	Du WD, Yuan ZR, Sun J, Tang JX, Cheng AQ, Shen DM, et al. Therapeutic efficacy of high-dose vitamin C on acute pancreatitis and its potential mechanisms. <i>World J Gastroenterol</i> 2003; 9(11): 2565-2569.
S63	Eaton S. The biochemical basis of antioxidant therapy in critical illness. <i>Proc Nutr Soc</i> 2006; 65(3): 242-249.
S64	Faure H, Peyrin JC, Richard MJ, Favier A. Parenteral supplementation with zinc in surgical patients corrects postoperative serum-zinc drop. <i>Biol Trace Elem Res</i> 1991; 30: 37-45.
S66	Forceville X, Vitoux D, Gauzit R, Combes A, Lahilaire P. Selenium, systemic immune response syndrome, sepsis, and outcome in critically ill patients. <i>Crit Care Med</i> 1998; 26: 1536-1544.
S67	Gadek JE, DeMichele SJ, Karlstad MD, Pacht ER, Donahoe M, Albertson TE, et al. Effect of enteral feeding with eicosapentaenoic acid, gamma-linolenic acid, and antioxidants in patients with acute respiratory distress syndrome. <i>Crit Care Med</i> 1999; 27(8): 1409-1420.
S69	García Garmendia JL, Garnacho Montero J, Ortiz Leyba C, Jiménez Jiménez FJ, Moyano del Estad MR, Monterrubio Villar J, et al. Cytokine levels in critically-ill septic patients fed with an enteral diet supplemented with fish oil and vitamin E. <i>Clin Nutr</i> 1998; 17(Suppl 1): 6.
S70	Gärtner R, Angstwurm M. Die Bedeutung von selen in der intensivmedizin [Significance of selenium in intensive care medicine. Clinical studies of patients with SIRS/sepsis syndrome]. <i>Med Klin</i> 1999; 94(Suppl 3): 54-57.
S71	Geoghegan M, McAuley D, Eaton S, Powell-Tuck J. Selenium in critical illness. <i>Curr Opin Crit Care</i> 2006; 12(2): 136-141.

Study ID	Study reference (excluded)
S72	Gogos CA, Ginopoulos P, Salsa B, Apostolidou E, Zoumbos NC, Kalfarentzos F. Dietary omega-3 polyunsaturated fatty acids plus vitamin E restore immunodeficiency and prolong survival for severely ill patients with generalized malignancy: a randomized control trial. <i>Cancer</i> 1998; 82(2): 395-402.
S73	Grau T, Caparros T, Lopez J. Early enteral nutrition in critically ill patients with a high-protein diet enriched with fiber and antioxidants compared with a standard high-protein diet. Effect on nosocomial infections and outcome. <i>J Parenter Enteral Nutr</i> 2001; 25(1): S1.
S74	Grimble RF. Effect of antioxidative vitamins on immune function with clinical applications. <i>Internat J Vit Nutr Res</i> 1997; 67: 312-320.
S75	Grimble RF. Modification of inflammatory aspects of immune function by nutrients. <i>Nutr Res</i> 1998; 18(7): 1297-1317.
S76	Heyland DK, Dhaliwal R, Day AG, Muscedere J, Drover J, Suchner U, et al. REducing Deaths due to OXidative Stress (The REDOXS Study): rationale and study design for a randomized trial of glutamine and antioxidant supplementation in critically-ill patients. <i>Proc Nutr Soc</i> 2006; 65: 250-263.
S77	Heyland DK, Dhaliwal R, Suchner U, Berger MM. Antioxidant nutrients: a systematic review of trace elements and vitamins in the critically ill patient. <i>Intensive Care Med</i> 2005; 31(3): 327-337.
S78	Heyland DK, Dhaliwal R, Day A, Drover J, Cote H, Wischmeyer P. Optimizing the dose of glutamine dipeptides and antioxidants in critically ill patients: a phase I dose-finding study. <i>J Parenter Enteral Nutr</i> 2007; 31(2): 109-118.
S79	Heyland DK. Selenium supplementation in critically ill patients: can too much of a good thing be a bad thing? <i>Crit Care</i> 2007; 11(4): 153.
S80	Kieft H, Roos AN, van Drunen JD, Bindels AJ, Bindels JG, Hofman Z. Clinical outcome of immunonutrition in a heterogeneous intensive care population. <i>Intensive Care Med</i> 2005; 31(4): 524-532.
S81	Kreymann KG, Berger MM, Deutz NEP, Hiesmayr M, Jolliet P, Kazandjiev G, et al. ESPEN guidelines on enteral nutrition: intensive care. <i>Clin Nutr</i> 2006; 25(2): 210-223.
S82	Kuklinski B, Buchner M, Muller T, Schweder R. [Anti-oxidative therapy of pancreatitis-an 18-month interim evaluation]. <i>Z Gesamte Inn Med</i> 1992; 47: 239-245.
S83	Kuklinski B, Buchner M, Schweder R, Nagel R. Akute Pancreatitis—eine free radical disease. Letalitatssenkung durch Natriumselenit (Na <sub>2</sub> SeO <sub>3</sub> )-Therapie [Acute pancreatitis—a "Free radical disease". Decreasing mortality by sodium selenite (Na <sub>2</sub> SeO <sub>3</sub> ) therapy]. <i>Z Gesamte Inn Med</i> 1991; 46: S145-S149.
S84	Kuklinski B, Zimmermann T, Schweder R. Letalitätssenkung der akuten Pankreatitis mit Natriumselenit [Decreasing mortality in acute pancreatitis with sodium selenite. Clinical results of 4 years antioxidant therapy]. <i>Med Klin</i> 1995; 90(Suppl 1): 36-41.
S85	Lafrance JP, Leblanc M. Metabolic, electrolytes, and nutritional concerns in critical illness. <i>Crit Care Clin</i> 2005; 21(2): 305-327.
S86	Lehmann C, Egerer K, Weber M, Krausch D, Wauer H, Newie T, et al. Einfluss einer Selensubstitution auf verschiedene Laborparameter bei sepsisgefährdeten Patienten [Effect of selenium administration on various laboratory parameters of patients at risk for sepsis syndrome]. <i>Med Klin</i> 1997; 92(Suppl 3): 14-16.
S87	Lehmann C, Weber M, Krausch D, Wauer H, Newie T, Rohr U, et al. Parenteral selenium supplementation in critically ill patients - effects on antioxidant metabolism. <i>Z Ernährungswiss</i> 1998; 37(Suppl 1): 106-109.
S88	Leichtle A, Teupser D, Thiery J. Alpha-tocopherol distribution in lipoproteins and anti-inflammatory effects differ between CHD-patients and healthy subjects. <i>J Am Coll Nutr</i> 2006; 25(5): 420-428.
S89	Levy J, Turkish A. Protective nutrients. <i>Curr Opin Gastroenterol</i> 2002; 18(6): 717-722.
S90	Long CL, Maull KI, Krishnan RS, Laws HL, Geiger JW, Borghesi L, et al. Ascorbic acid dynamics in the seriously ill and injured. <i>J Surg Res</i> 2003; 109: 144-148.

Study ID	Study reference (excluded)
S91	Lovat R, Preiser JC. Antioxidant therapy in intensive care. <i>Curr Opin Crit Care</i> 2003; 9: 266-270.
S93	Manzanares Castro W. Selenio en los pacientes críticos con respuesta inflamatoria sistémica [Selenium in critically ill patients with systemic inflammatory response]. <i>Nutr Hosp</i> 2007; 22(3): 295-306.
S94	Mashour S, Turner JF Jr, Merrell R. Acute renal failure, oxalosis, and vitamin C supplementation: a case report and review of the literature. <i>Chest</i> 2000; 118: 561-563.
S95	Matsuda T, Tanaka H, Hanumadass M, Gayle R, Yuasa H, Abcarian H, et al. Effects of high-dose vitamin C administration on postburn microvascular fluid and protein flux. <i>J Burn Care Rehabil</i> 1992; 13: 560-566.
S96	McConachie I, Haskew A. Thiamine status after major trauma. <i>Intensive Care Med</i> 1988; 14(6): 628-631.
S97	Mechanick JL, Brett EM. Nutrition support of the chronically critically ill patient. <i>Crit Care Clin</i> 2002; 18(3): 597-618.
S98	Mendez C, Jurkovich GJ, Garcia I, Davis D, Parker A, Maier RV. Effects of an immune-enhancing diet in critically injured patients. <i>J Trauma</i> 1997; 42(5): 933-941.
S99	Meydani SN, Beharka AA. Recent developments in vitamin E and immune response. <i>Nutr Rev</i> 1998; 56(1 Pt 2): S49-S58.
S100	Meyer NA, Muller MJ, Herndon DN. Nutrient support of the healing wound. <i>New Horiz</i> 1994; 2(2): 202-214.
S101	Mingjian Z, Qifang W, Lanxing G, Hong J, Zongyin W. Comparative observation of the changes in serum lipid peroxides influenced by the supplementation of vitamin E in burn patients and healthy controls. <i>Burns</i> 1992; 18(1): 19-21.
S102	Mishra V, Baines M, Perry S, McLaughlin J, Carson J, Wenstone R, et al. Selenium supplementation and outcome in septic ICU patients. <i>Clin Chim Acta</i> 2005; 355: S45-S46.
S104	Mishra V, Baines M, Wenstone R, Shenkin A. Markers of oxidative damage, antioxidant status, and clinical outcome in critically ill patients. <i>Ann Clin Biochem</i> 2005; 42: 269-276.
S105	Mishra V. Oxidative stress and role of antioxidant supplementation in critical illness. <i>Clin Lab</i> 2007; 53(3-4): 199-209.
S106	Murray MJ, Murray AB, Murray MS, Murray CJ. The adverse effect of iron repletion on the course of certain infections. <i>Br Med J</i> 1978; 2(6145): 1113-1115.
S108	Nelson JL, DeMichele SJ, Pacht ER, Wennberg AK; Enteral Nutrition in ARDS Study Group. Effect of enteral feeding with eicosapentanoic acid, gamma-linolenic and antioxidants on antioxidant status in patients with acute respiratory distress syndrome. <i>J Parenter Enteral Nutr</i> 2003; 27: 98-104.
S109	Nitenberg G. Apports nutritionnels en réanimation [Nutritional supply in the critically ill]. <i>Réanimation</i> 2003; 12(5): 340-349.
S110	Ochoa JB. Separating pharmaconutrition from classic nutrition goals: a necessary step. <i>Crit Care Med</i> 2008; 36(1): 347-348.
S111	Oldham KM, Bowen PE. Oxidative stress in critical care: is antioxidant supplementation beneficial? <i>J Am Diet Assoc</i> 1998; 98(9): 1001-1008.
S112	Pacht ER, DeMichele SJ, Nelson JL, Hart J, Wennberg AK, Gadek JE. Enteral nutrition with eicosapentaenoic acid, gamma-linolenic acid, and antioxidants reduces alveolar inflammatory mediators and protein influx in patients with acute respiratory distress syndrome. <i>Crit Care Med</i> 2003; 31(2): 491-500.
S113	Pingleton SK. Nutrition in chronic critical illness. <i>Clin Chest Med</i> 2001; 22(1): 149-163.
S114	Pontes-Arruda A, Aragão AM, Albuquerque JD. Effects of enteral feeding with eicosapentaenoic acid, gamma-linolenic acid, and antioxidants in mechanically ventilated patients with severe sepsis and septic shock. <i>Crit Care Med</i> 2006; 34(9): 2325-2333.

Study ID	Study reference (excluded)
S116	Powell-Tuck J. Nutritional interventions in critical illness. <i>Proc Nutr Soc</i> 2007; 66(1): 16-24.
S117	Preiser JC, Berre J, Van Gossum A, Vincent JL, Carpentier YA. Effects of enteral feeding supplementation with arginine and vitamins A, C and E in critically ill patients. <i>J Parenter Enteral Nutr</i> 1998; 22(1): S4.
S119	Prelack K, Dylewski M, Sheridan RL. Practical guidelines for nutritional management of burn injury and recovery. <i>Burns</i> 2007; 33(1): 14-24.
S120	Prelack K, Sheridan RL. Micronutrient supplementation in the critically ill patient: strategies for clinical practice. <i>J Trauma</i> 2001; 51(3): 601-620.
S121	Quasim T, McMillan DC, Talwar D, Vasilaki A, St.J.O'Reilly D, Kinsella J. The relationship between plasma and red cell B-vitamin concentrations in critically-ill patients. <i>Clin Nutr</i> 2005; 24(6): 956-960.
S122	Rabe C, Gramann T, Sons X, Berna M, González-Carmona MA, Klehr HU, et al. Keeping central venous lines open: a prospective comparison of heparin, vitamin C and sodium chloride sealing solutions in medical patients. <i>Intensive Care Med</i> 2002; 28(8): 1172-1176.
S123	Rabl H, Khoschsorur G, Petek W. Antioxidative vitamin treatment: effect on lipid peroxidation and limb swelling after revascularization operations. <i>World J Surg</i> 1995; 19(5): 738-744.
S125	Roth E, Manhart N, Wessner B. Assessing the antioxidative status in critically ill patients. <i>Curr Opin Clin Nutr Metab Care</i> 2004; 7(2): 161-168.
S126	Rotstein OD. Oxidants and antioxidant therapy. <i>Crit Care Clin</i> 2001; 17(1): 239-248.
S127	Rümelin A, Dörr, S, Depta A, Fauth U. Preoperative oral ascorbic acid (AA) and postoperative plasma levels of AA. <i>Clin Nutr</i> 2001; 20(Suppl 3): 47.
S129	Sacks GS. Randomized, prospective trial of antioxidant supplementation in critically ill surgical patients [comment]. <i>Nutr Clin Pract</i> 2003; 18(3): 264.
S130	Schomburg L. Selenium in intensive care (SIC) study: the XX files are still unresolved. <i>Crit Care Med</i> 2007; 35(3): 995-996; author reply 996-997.
S131	Seeger W, Ziegler A, Wolf HRD. Serum alpha-tocopherol levels after high-dose enteral vitamin E administration in patients with acute respiratory failure. <i>Intensive Care Med</i> 1987; 13: 395-400.
S132	Shenkin A. Micronutrients and outcome. <i>Nutrition</i> 1997; 13(9): 825-828.
S133	Shenkin A. The key role of micronutrients. <i>Clin Nutr</i> 2006; 25(1): 1-13.
S134	Singer P, Theilla M, Fisher H, Gibstein L, Grozovski E, Cohen J. Benefit of an enteral diet enriched with eicosapentaenoic acid and gamma-linolenic acid in ventilated patients with acute lung injury. <i>Crit Care Med</i> 2006; 34(4): 1033-1038.
S136	Slone DS. Nutritional support of the critically ill and injured patient. <i>Crit Care Clin</i> 2004; 20(1): 135-157.
S137	Soeters PB, DeJong CHC, Deutz NEP. Clinical nutrition: the future. <i>Clin Nutr</i> 2001; 20(Suppl 1): 191-197.
S138	Stawicki SP, Lyons M, Aloupis M, Sarani B. Current evidence from phase III clinical trials of selenium supplementation in critically ill patients: why should we bother? <i>Mini Rev Med Chem</i> 2007; 7(7): 693-699.
S139	Tanaka H, Matsuda T, Miyagantani Y, Yukioka T, Matsuda H, Shimazaki S. Reduction of resuscitation fluid volumes in severely burned patients using ascorbic acid administration: a randomized, prospective study. <i>Arch Surg</i> 2000; 135(3): 326-331.
S140	Theilla M, Singer P, Cohen J, DeKeyser F. A diet enriched in eicosapentanoic acid, gamma-linolenic acid and antioxidants in the prevention of new pressure ulcer formation in critically ill patients with acute lung injury: a randomized, prospective, controlled study. <i>Clin Nutr</i> 2007; 26(6): 752-757.
S141	Ullegaddi R, Powers HJ, Gariballa SE. Antioxidant supplementation with or without B-group vitamins after acute ischemic stroke: a randomized controlled trial. <i>J Parenter Enteral Nutr</i> 2006; 30(2): 108-114.

Study ID	Study reference (excluded)
S142	Ullegaddi R, Powers HJ, Gariballa SE. B-group vitamin supplementation mitigates oxidative damage after acute ischaemic stroke. <i>Clin Sci (Lond)</i> 2004; 107: 477-484.
S143	Van Iperen CE, Gaillard CA, Kraaijenhagen RJ, Braam BG, Marx JJ, Van de Wiel A. Response of erythropoiesis and iron metabolism to recombinant human erythropoietin in intensive care unit patients. <i>Crit Care Med</i> 2000; 28(8): 2773-2778.
S144	Vaxman F, Olender S, Lambert A, Nisand G, Grenier JF. Can the wound healing process be improved by vitamin supplementation? <i>Eur Surg Res</i> 1996; 28: 306-314.
S145	Wheatley C. A scarlet pimpernel for the resolution of inflammation? The role of supra-therapeutic doses of cobalamin, in the treatment of systemic inflammatory response syndrome (SIRS), sepsis, severe sepsis, and septic or traumatic shock. <i>Med Hypotheses</i> 2006; 67(1): 124-142.
S147	Zanello M, Di Mauro L, Vincenzi M. Therapeutic effects of artificial nutrition in intensive care patients: new insights. <i>Curr Anaesth Crit Care</i> 2006; 17(6): 375-383.
S148	Zang Q, Maass DL, White J, Horton JW. Cardiac mitochondrial damage and loss of ROS defense after burn injury: the beneficial effects of antioxidant therapy. <i>J Appl Physiol</i> 2007; 102(1): 103-112.
S149	Zazzo JF. Stress oxydant au cours des états inflammatoires aigus et des états d'agression: implications pour la pratique clinique [Oxidative stress during acute inflammatory and critical states: implications for clinical practice]. <i>Nutr Clin Métabol</i> 2002; 16(4): 268-274.
S150	Ziegler TR, Leader LM, Jonas CR, Griffith DP. Adjunctive therapies in nutritional support. <i>Nutrition</i> 1997; 13(9 Suppl): S64-S72.
S151	Zimmermann T, Albrecht S, Kühne H, Vogelsang U, Grützmann R, Kopprasch S. Selensubstitution bei Sepsispatienten. Eine prospektiv randomisierte Studie [Selenium administration in patients with sepsis syndrome. A prospective randomized study]. <i>Med Klin</i> 1997; 92(Suppl 3): 3-4.

**Appendix 6.5: Bibliographic information, study identification numbers and source(s) of the included studies (\* Reference number in brackets)**

STUDY ID (*)	STUDY	SOURCE(S)
S3 (156)	Angstwurm MW, Engelmann L, Zimmermann T, Lehmann C, Spes CH, Abel P, et al. Selenium in Intensive Care (SIC): results of a prospective randomized, placebo-controlled, multiple-center study in patients with severe systemic inflammatory response syndrome, sepsis, and septic shock. <i>Crit Care Med</i> 2007; 35(1): 118-126.	ISI Web of Science
S5 (157)	Angstwurm MW, Schopohl J, Gaertner R. Selenium substitution has no direct effect on thyroid hormone metabolism in critically ill patients. <i>Eur J Endocrinol</i> 2004; 151(1): 47-54.	Medline; Cochrane Clinical Trials
S6 (158)	Angstwurm MW, Schottdorf J, Schopohl J, Gaertner R. Selenium replacement in patients with severe systemic inflammatory response syndrome improves clinical outcome. <i>Crit Care Med</i> 1999; 27(9): 1807-1813.	Reference lists / Personal files
S14 (159)	Berger MM, Baines M, Chioléro RL, Wardle CA, Cayeux C, Shenkin A. Influence of early trace element and vitamin E supplements on antioxidant status after major trauma: a controlled trial. <i>Nutr Res</i> 2001; 21: 41-54.	Science Direct; Cochrane Clinical Trials
S15 (160)	Berger MM, Baines M, Raffoul W, Benathan M, Chioléro RL, Reeves C, et al. Trace element supplementation after major burns modulates antioxidant status and clinical course by way of increased tissue trace element concentrations. <i>Am J Clin Nutr</i> 2007; 85(5): 1293-1300.	Medline; ISI Web of Science
S18 (161)	Berger MM, Binnert C, Chioléro RL, Taylor W, Raffoul W, Cayeux MC, et al. Trace element supplementation after major burns increases burned skin trace element concentrations and modulates local protein metabolism but not whole-body substrate metabolism. <i>Am J Clin Nutr</i> 2007; 85(5): 1301-1306.	ISI Web if Science
S26 (162)	Berger MM, Reymond MJ, Shenkin A, Rey F, Wardle C, Cayeux C, et al. Influence of selenium supplements on the post-traumatic alterations of the thyroid axis: a placebo-controlled trial. <i>Intensive Care Med</i> 2001; 27: 91-100.	Medline
S33 (163)	Berger MM, Spertini F, Shenkin A, Wardle C, Wiesner L, Schindler C, et al. Trace element supplementation modulates pulmonary infection rates after major burns: a double-blind, placebo-controlled trial. <i>Am J Clin Nutr</i> 1998; 68: 365-371.	Medline
S52 (164)	Cheng CH, Chang SJ, Lee BJ, Lin KL, Huang YC. Vitamin B6 supplementation increases immune responses in critically ill patients. <i>Eur J Clin Nutr</i> 2006; 60(10): 1207-1213.	Medline; Cochrane Clinical Trials; Proquest Medical Library
S54 (165)	Crimi E, Liguori A, Condorelli M, Cioffi M, Astuto M, Bontempo P, et al. The beneficial effects of antioxidant supplementation in enteral feeding in critically ill patients: a prospective, randomized, double-blind, placebo-controlled trial. <i>Anesth Analg</i> 2004; 99(3): 857-863.	Medline; Cochrane Clinical Trials
S65 (166)	Forceville X, Laviolle B, Annane L, Vitoux D, Bleichner G, Korach JM, et al. Effects of high doses of selenium, as sodium selenite, in septic shock: a placebo-controlled, randomized, double-blind, phase II study. <i>Crit Care</i> 2007; 11(4): R73.	Proquest Medical Library; ISI Web of Science

STUDY ID (*)	STUDY	SOURCE(S)
S68 (167)	Galley HF, Howdle PD, Walker BE, Webster NR. The effects of intravenous antioxidants in patients with septic shock. <i>Free Radic Biol Med</i> 1997; 23(5): 768-774.	Reference lists / Personal files
S92 (168)	Maderazo EG, Woronick CL, Hickingbotham N, Jacobs L, Bhagavan HN. A randomized trial of replacement antioxidant vitamin therapy for neutrophil locomotory dysfunction in blunt trauma. <i>J Trauma</i> 1991; 31(8): 1142-1150.	Medline; Cochrane Clinical Trials
S103 (169)	Mishra V, Baines M, Perry SE, McLaughlin PJ, Carson J, Wenstone R, et al. Effect of selenium supplementation on biochemical markers and outcome in critically ill patients. <i>Clin Nutr</i> 2007; 26(1): 41-50.	Science Direct
S107 (170)	Nathens AB, Neff MJ, Jurkovich GJ, Klotz P, Farver K, Ruzinski JT, et al. Randomized, prospective trial of antioxidant supplementation in critically ill surgical patients. <i>Ann Surg</i> 2002; 236(6): 814-822.	Medline; Cochrane Clinical Trials
S115 (171)	Porter JM, Ivatury RR, Azimuddin K, Swami R. Antioxidant therapy in the prevention of organ dysfunction syndrome and infectious complications after trauma: early results of a prospective randomized study. <i>Am Surg</i> 1999; 65(5): 478-483.	Medline; Cochrane Clinical Trials
S118 (172)	Preiser JC, Van Gossum A, Berré J, Vincent JL, Carpentier Y. Enteral feeding with a solution enriched with antioxidant vitamins A, C, and E enhances the resistance to oxidative stress. <i>Crit Care Med</i> 2000; 28(12): 3828-3832.	Medline; Cochrane Clinical Trials
S124 (173)	Rock CL, Dechert RE, Khilnani R, Parker RS, Rodriguez JL. Carotenoids and antioxidant vitamins in patients after burn injury. <i>J Burn Care Rehabil</i> 1997; 18(3): 269-278.	Medline; Cochrane Clinical Trials
S128 (174)	Rümelin A, Jaehde U, Kerz T, Roth W, Krämer M, Fauth U. Early postoperative substitution procedure of the antioxidant ascorbic acid. <i>J Nutr Biochem</i> 2005; 16(2): 104-108.	Medline; Cochrane Clinical Trials
S135 (175)	Siriwardena AK, Mason JM, Balachandra S, Bagul A, Galloway S, Formela L, et al. Randomized, double blind, placebo controlled trial of intravenous antioxidant (n-acetylcysteine, selenium, vitamin C) therapy in severe acute pancreatitis. <i>Gut</i> 2007; 56: 1439-1444.	Medline
S146 (176)	Young B, Ott L, Kasarskis E, Rapp R, Moles K, Dempsey RJ, et al. Zinc supplementation is associated with improved neurologic recovery rate and visceral protein levels of patients with severe closed head injury. <i>J Neurotrauma</i> 1996; 13(1): 25-34.	Reference lists / Personal files

Appendix 6.6: Characteristics of included trials

Study (& Study ID)	Population	Sex	Age	Route	Timing of Intervention	Intervention	Outcomes
Angstwurm 2007 (S3)	Patients with SIRS, sepsis and septic shock, and APACHE III >70 (n=249)	M: 162 F: 76	64.6 years (SD 14.0)  Se1: 63.9±13.8 Se0: 65.3±14.1	IV	Admission into study after diagnosis within 24 hours, study treatment beginning within 1 hour after inclusion, up until 14 days	<p><b>a: Se1:</b> 48ml vial as bolus intravenous injection over 30 minutes of sodium-selenite providing 1000 µg selenium, followed by continuous infusion of 2 ml/hour over 24 hours for 14 days, total dose 15 000 µg selenium. Allowed selenium from other preparations of up to 100 µg/day</p> <p><b>b: Se0:</b> Matching placebo of 0.9% sodium chloride give as same regimen. Allowed selenium from other preparations of up to 100 µg/day</p> <p>EN/PN (including standard MN) in all patients as appropriate</p> <p>Allocated: ???/??? Assessed: 116/122 (11 patients excluded after randomization: n=238)</p>	<p>Follow-up: 28 days</p> <p><u>Main study endpoints:</u></p> <ul style="list-style-type: none"> <li>• Clinical outcome</li> <li>• Mortality (28 days)</li> </ul> <p><u>Outcomes (related to study objectives):</u></p> <ul style="list-style-type: none"> <li>• Mortality (28 days)</li> <li>• Infections</li> <li>• LICU</li> <li>• Morbidity (APACHE III)</li> <li>• MN status (Se)</li> <li>• CRP</li> <li>• GPx-3</li> </ul>

Study (& Study ID)	Population	Sex	Age	Route	Timing of Intervention	Intervention	Outcomes
Angstwurm 2004 & 1999* (S5 & S6*)	Patients with SIRS (n=42)	M: 29 F: 13	Mean age 56 years (range 18-83)  Se+: 54.3±4.9 Se-: 58.5±5.2	IV	From day of admission to ICU (within 24 hours) for additional supplementation for 9 days	<p><b>a: Se+:</b> Continuous intravenous sodium selenite (535 µg selenium for 3 days, 285 µg selenium for 3 days, 155 µg selenium for 3 days, 35 µg selenium for remainder of total treatment time; and standard parenteral nutrition including glutamine 20 g/L</p> <p><b>b: Se-:</b> Continuous placebo of saline and intravenous 35 µg selenium as sodium selenite, and standard parenteral nutrition including glutamine 20 g/L</p> <p>Allocated: 21/21 Assessed: 21/21</p>	<p>Follow-up: until discharge (max 90 days) Study period: 14 days</p> <p><u>Main study endpoints:</u></p> <ul style="list-style-type: none"> <li>• Acute renal failure, ICU Outcome &amp; Thyroid metabolism</li> </ul> <p><u>Outcomes (related to study objectives):</u></p> <ul style="list-style-type: none"> <li>• Mortality</li> <li>• LICU</li> <li>• LOS (survivors)</li> <li>• Morbidity (APACHE III)</li> <li>• MN status (Se)</li> <li>• GSH-Px</li> </ul>

Study (& Study ID)	Population	Sex	Age	Route	Timing of Intervention	Intervention	Outcomes
Berger 2001 (S14* & S26)	Trauma patients, surgical ICU (n=32)	M: 23 F: 9	Mean age 42 years (range 18-74)  Se <sup>only</sup> : 43±13 (24-63) Se <sup>plus</sup> : 40±14 (20-64) Placebo: 44±21 (18-74)	IV	From day of admission (within 2 hours) for 5 days	<p><b>a: Se<sup>only</sup>:</b> Slow intravenous infusion over 24 hours of 500 µg selenium as sodium selenite/day</p> <p><b>b: Se<sup>plus</sup>:</b> Slow intravenous infusion over 24 hours of 500 µg selenium/day, 2.6 mg copper/day and 13 mg zinc/day, 150 mg α-tocopherol in 5 ml 10% lipid emulsion as slow injection once daily upon initiation of intravenous infusion</p> <p><b>c: Placebo:</b> Infusion vehicle over 24 hours</p> <p>All groups received EN</p> <p>Allocated: 9/11/12 Assessed: 9/11/12 for primary objectives (9/11/11 for S26)</p>	<p>Follow-up: hospital discharge or died (max 249 days) Study period: 20 days</p> <p><u>Main study endpoints:</u></p> <ul style="list-style-type: none"> <li>• AOX status &amp; Thyroid function</li> </ul> <p><u>Outcomes (related to study objectives):</u></p> <ul style="list-style-type: none"> <li>• Mortality</li> <li>• Infections</li> <li>• LICU</li> <li>• LOS</li> <li>• MN status (Se, Cu, Zn, α-tocopherol)</li> <li>• Leukocytes</li> <li>• CRP</li> <li>• TAC</li> <li>• GSHPx</li> </ul>

Study (& Study ID)	Population	Sex	Age	Route	Timing of Intervention	Intervention	Outcomes
Berger 2007 (S15* & S18)	Burns >20% TBSA (n=21)	M: 15 F: 6	42.5±15.8  TE group: 46±15 Vehicle (V) group: 38±16	IV	Treatment within 12 h of injury and for 14 d if burned on 20–60% BSA (n=16) or for 21 d if burns >60% BSA (n=5)	<p><b>a: TE group:</b> 250 ml of a 0.9% saline solution over 12 h containing 59 µmol (3.75 mg) Cu, 4.8 µmol (375 µg) Se, and 574 µmol (37.5 mg) Zn per day IV (provided as copper gluconate, sodium selenite and zinc gluconate)</p> <p><b>b: V group:</b> 250 ml of a 0.9% saline solution over 12 h containing the vehicle</p> <p>EN (containing normal amounts of TE) started within 16h in all but 1 patient who was fed PO. Both groups: daily vitamin supplements: 1 g ascorbic acid, 100 mg thiamine, and multivitamin preparation delivered IV (1 ampoule Soluvit &amp; Vitalipid) + 100 mg α-tocopherol delivered via feeding tube</p> <p>Allocated: 11/10 Assessed: 11/10 for mortality, infections &amp; LICU</p>	<p>Follow-up: discharge from hospital Study period: 21 days</p> <p><u>Main study endpoints:</u></p> <ul style="list-style-type: none"> <li>• TE concentrations</li> <li>• AOX status</li> <li>• Clinical outcome</li> <li>• Systemic substrate turnover &amp; local protein metabolism (wound healing)</li> </ul> <p><u>Outcomes (related to study objectives):</u></p> <ul style="list-style-type: none"> <li>• Mortality</li> <li>• Infections</li> <li>• LICU</li> <li>• LOS?? (mentioned; not reported)</li> <li>• MN status (Se, Cu, Zn, Retinol, α-tocopherol)</li> <li>• CRP</li> <li>• Cytokines (IL-6, IL-10)</li> <li>• GSHPx</li> </ul>

Study (& Study ID)	Population	Sex	Age	Route	Timing of Intervention	Intervention	Outcomes
Berger 1998 (S33)	Burns >30% TBSA (n=20)	NA	40 years (SD 16) (range 21-61)  TE group: 39±16 (21-61) Control group: 43±14 (26-61)	IV	From day of admission (within 1.5 hours) for 8 days	<p><b>a: TE group:</b> Standard trace element intakes (IV as a separate infusion) plus IV supplements [total copper (40.4 µmol/2.5 mg), selenium (2.9 µmol/315 µg), zinc (406 µmol/26.2 mg) in a 0.9% NaCl infusion; given as copper gluconate, sodium selenite, and zinc gluconate]</p> <p><b>b: Control group:</b> IV placebo 0.9% NaCl infusion and standard trace elements [total copper 20 µmol, selenium 0.4 µmol (32 µg), zinc 100 µmol]</p> <p>All received early EN (within 12 h of admission)</p> <p>Allocated: 10/10 Assessed: 10/10</p>	<p>Follow-up: hospital discharge or died (max 129 days) Study period: 30 days</p> <p><u>Main study endpoints:</u></p> <ul style="list-style-type: none"> <li>• Infections</li> <li>• LOS</li> </ul> <p><u>Outcomes (related to study objectives):</u></p> <ul style="list-style-type: none"> <li>• Mortality</li> <li>• Infections</li> <li>• LICU</li> <li>• LOS</li> <li>• MN status (Cu, Se and Zn)</li> <li>• Cytokines (IL-6)</li> <li>• Leukocytes &amp; neutrophils</li> <li>• CRP</li> </ul>

Study (& Study ID)	Population	Sex	Age	Route	Timing of Intervention	Intervention	Outcomes
Cheng 2006 (S52)	Surgical/medical ICU (n=51) (37 surgical; 14 medical)	M: 41 F: 10	70.2 years (mean) (range 24-91)  B <sub>6</sub> -100: 71.7±17.2 B <sub>6</sub> -50: 65.4±11.8 Control: 73.0±14.1	IV	Unable to determine exact timing of intervention: after study inclusion daily injection for 14 days	<p><b>a: B<sub>6</sub>-100 group:</b> 100mg vitamin B6 [as vitamin B6 injection (pyridoxine HCl)]</p> <p><b>b: B<sub>6</sub>-50 group:</b> 50mg vitamin B6 [as vitamin B6 injection (pyridoxine HCl)]</p> <p><b>c: Control:</b> No vitamin B6 injection</p> <p>EN and/or PN in all patients (based on physician recommendations)</p> <p>Allocated: 15/16/20 Assessed: 15/16/20</p>	<p>Follow-up &amp; study period: 14 days</p> <p><u>Main study endpoints:</u></p> <ul style="list-style-type: none"> <li>• Immune responses</li> </ul> <p><u>Outcomes (related to study objectives):</u></p> <ul style="list-style-type: none"> <li>• No primary objectives covered</li> <li>• Morbidity (APACHE II)</li> <li>• MN status (PLP)</li> <li>• WBC / Neutrophils / Leukocytes</li> <li>• CRP</li> </ul>

Study (& Study ID)	Population	Sex	Age	Route	Timing of Intervention	Intervention	Outcomes
Crimi 2004 (S54)	Coronary critical care/ medico-surgical ICUs (n=224)	M: 147 F: 69	61.5 years (average)  AOX protocol: 61.9±7.0 Regular diet: 61.0±7.3	EN	Unable to determine exact timing of intervention: after study inclusion for 10 days (trauma patients enrolled within 48 h after injury; other patients enrolled within 72 h of ICU admission)	<p><b>a: AOX protocol:</b> AOX supplementation with 500mg/d vitamin C and 400 IU/d vitamin E in enteral feeding preparation</p> <p><b>b: Regular diet:</b> Equal amount of isotonic saline solution in enteral feeding preparation</p> <p>All received EN</p> <p>Allocated: 112/112 Assessed: 112/112 for some analyses: mortality, LOS; 105/111 (for other analyses) (8 patients excluded after randomization: n=216)</p>	<p>Follow-up: until discharge Study period: 10 days</p> <p><u>Main study endpoints:</u></p> <ul style="list-style-type: none"> <li>• Variables of oxidative stress</li> <li>• Clinical outcome</li> </ul> <p><u>Outcomes (related to study objectives):</u></p> <ul style="list-style-type: none"> <li>• Mortality (28 day)</li> <li>• Infections</li> <li>• LOS</li> <li>• MN status (Plasma &amp; LDL bound tocopherol levels)</li> <li>• Malonyldialdehyde measured with TBARS</li> <li>• F<sub>2α</sub> isoprostanes</li> <li>• LDL resistance to oxidative stress (lag time)</li> </ul>

Study (& Study ID)	Population	Sex	Age	Route	Timing of Intervention	Intervention	Outcomes
Forceville 2007 (S65)	Patients with septic shock (n=60)	M: 38 F: 22	Se group: 66±14 Placebo group: 69±12 (mean±SD)	IV	Treatment started day 1 of study inclusion (majority of patients included within 48 h of ICU admission) for 10 days	<p><b>a: Se group:</b> Patients received, for 10 days, selenium as sodium selenite (4,000 µg: first day, 1,000 µg/day: nine following days) using continuous intravenous infusion (2 ml/hour)</p> <p><b>b: Placebo group:</b> Matching placebo: continuous intravenous infusion (2 ml/hour), day 1-10</p> <p>No further information re nutrition support/care</p> <p>Allocated: 31/29 Assessed: 31/29</p>	<p>Follow-up &amp; study period: 1 year or death (depending on which occurred first)</p> <p><u>Main study endpoints:</u></p> <ul style="list-style-type: none"> <li>• Vasopressor therapy withdrawal</li> </ul> <p><u>Outcomes (related to study objectives):</u></p> <ul style="list-style-type: none"> <li>• Mortality (28 day / 6 months / 1 year)</li> <li>• Infections</li> <li>• LICU</li> <li>• LOS</li> </ul>

Study (& Study ID)	Population	Sex	Age	Route	Timing of Intervention	Intervention	Outcomes
Galley 1997 (S68)	Patients with septic shock (n=30)	M: 22 F: 8	Range: 21-89  AO group: 67 (21-78) Placebo group: 70 (22-89) [median (range)]	IV	Treatment immediately after enrollment for 6 hours	<p><b><u>a: AO group:</u></b> IV AO therapy comprising <i>n</i>-acetylcysteine 150 mg/kg for 30 min then 20 mg/kg/h plus bolus doses of 1 g ascorbic acid (vitamin C) and 400 mg <math>\alpha</math>-tocopherol (vitamin E) given after the initial loading dose of <i>n</i>-acetylcysteine</p> <p><b><u>b: Placebo group:</u></b> Comparable volume of 5% dextrose as placebo</p> <p>No further information re nutrition support/care</p> <p>Allocated: 16/14 Assessed: 16/14</p>	<p>Follow-up: 6 hrs for main study outcomes; till ICU discharge (mortality)</p> <p><u>Main study endpoints:</u></p> <ul style="list-style-type: none"> <li>• Hemodynamic parameters</li> <li>• AO status and lipid peroxidation</li> </ul> <p><u>Outcomes (related to study objectives):</u></p> <ul style="list-style-type: none"> <li>• Mortality (ICU)</li> <li>• MN status (vitamin C)</li> <li>• Total lipid peroxide</li> <li>• Malonyldialdehyde</li> <li>• Nitrates</li> <li>• TAC</li> </ul>

Study (& Study ID)	Population	Sex	Age	Route	Timing of Intervention	Intervention	Outcomes
Maderazo 1991 (S92)	Blunt trauma (n=46)	M: 36 F: 10	Range: 14-62  $\alpha$ -T & AA group: 22 (median) (15-58 range) $\alpha$ -T group: 29 (18-62) AA group: 23 (19-30) Placebo group: 22.5 (14-50)	IV	Given as 2 h infusions from day 1-7 (Some patients only started treatment on day 2)	4 Groups: <b><u>a: <math>\alpha</math>-T &amp; AA group:</u></b> 200 mg ascorbic acid, then $\uparrow$ 500 mg + 50 mg $\alpha$ -tocopherol in 100 ml D5W Experimental group divided into two groups: <b><u>b: <math>\alpha</math>-T group:</u></b> 50 mg $\alpha$ -tocopherol infused in D5W <b><u>c: AA group:</u></b> 200 mg ascorbic acid infused in D5W <b><u>d: Placebo:</u></b> 100 ml of D5W  All groups received EN or oral intake  Allocated: 14/9/5/18 Assessed: 14/9/5*/18 (* for some determinations only 4 patients assessed)	Follow-up and Study period: 7 days  <u>Main study endpoints:</u> • Neutrophil locomotory  <u>Outcomes (related to study objectives):</u> • Infections  • MN status (AA & $\alpha$ -tocopherol)

Study (& Study ID)	Population	Sex	Age	Route	Timing of Intervention	Intervention	Outcomes
Mishra 2007 (S103)	Septic ICU patients (n=40)	M: 19 F: 21	66 years (mean)  Se+: 65.8 (11.2) Se-: 67.1 (10.2) [mean (SD)]	IV	Within 24 hours of admission to ICU and within 72 hours since diagnosis of sepsis, given until discharged	<p><b>a: Se+:</b> IV selenium (as selenium selenite) 474 µg/d for 3 days, then 316 µg/d for 3 days, then 158 µg/d for 3 days, and 31.6 µg/d thereafter</p> <p><b>b: Se-:</b> 31.6 µg/d</p> <p>EN/PN in all patients according to ICU nutrition guidelines</p> <p>Allocated 18/22 Assessed 18/22 for mortality, infections and LICU</p>	<p>Follow-up and study period: 28 days</p> <p><u>Main study endpoints:</u></p> <ul style="list-style-type: none"> <li>• Mortality (28 days)</li> <li>• Se status</li> <li>• Oxidative stress</li> <li>• Thyroid function</li> <li>• RRT</li> </ul> <p><u>Outcomes (related to study objectives):</u></p> <ul style="list-style-type: none"> <li>• Mortality (28 days)</li> <li>• Infections</li> <li>• LICU</li> <li>• MN status (Se)</li> <li>• CRP</li> <li>• GSH-Px / RBC GSH-Px</li> <li>• F2 isoprostanes</li> </ul>

Study (& Study ID)	Population	Sex	Age	Route	Timing of Intervention	Intervention	Outcomes
Nathens 2002 (S107)	General surgical, trauma ICU (n=770)	M: 452 F: 143	AO group: 38±15 No AO group: 39±15	IV and EN	Unable to determine exact timing of intervention (Treatment started following assignment for up to shorter duration of ICU admission or 28 days)	<p><b>a: AO group:</b> α-tocopherol (as dl-α-tocopheryl acetate) 1000 IU 20 ml q8h via naso- or orogastric tube and 1000 mg ascorbic acid given IV in 100ml D5W q8h</p> <p><b>b: No AO group:</b> Standard care</p> <p>All received standard care (EN and PN as appropriate)</p> <p>Allocated: ???/???</p> <p>Assessed: 301/294 (175 patients excluded after randomization: n=595)</p>	<p>Follow-up: Shorter of the duration of hospital admission or 28 days</p> <p><u>Main study endpoints:</u></p> <ul style="list-style-type: none"> <li>• Pulmonary morbidity</li> <li>• Multiple organ failure</li> </ul> <p><u>Outcomes (related to study objectives):</u></p> <ul style="list-style-type: none"> <li>• Mortality</li> <li>• Infections</li> <li>• LICU</li> <li>• LOS</li> <li>• MN status (α-tocopherol &amp; AA)</li> <li>• Cytokines (TNF-α, IL-1β, IL-6, IL-8)</li> <li>• WBC</li> <li>• F<sub>2α</sub> isoprostane</li> </ul>

Study (& Study ID)	Population	Sex	Age	Route	Timing of Intervention	Intervention	Outcomes
Porter 1999 (S115)	Surgical ICU, penetrating trauma patients with injury severity score $\geq$ 25 (n=18)	M: 18 F: 0	AO group: 30.1 Control group: 34.7 (means)	IV and EN or PO	From day 1 (within 8 hours of injury) -7	<p><b>a: AO group:</b> 50 <math>\mu</math>g selenium IV q 6 h + 400 IU vitamin E PO or via nasogastric (NG) tube q 8 h + 100 mg vitamin C PO or via NG tube q 8 h, and 8 g N-acetylcysteine (NAC) PO or via NG tube q 6 h</p> <p><b>b: Control:</b> None of the above</p> <p>No further information re nutrition support/care</p> <p>Allocated: 9/9 Assessed: 9/9</p>	<p>Follow-up: hospital discharge or died</p> <p><u>Main study endpoints:</u></p> <ul style="list-style-type: none"> <li>• Organ failure</li> <li>• Infections</li> </ul> <p><u>Outcomes (related to study objectives):</u></p> <ul style="list-style-type: none"> <li>• Mortality</li> <li>• Infections</li> <li>• LICU</li> <li>• LOS</li> </ul>

Study (& Study ID)	Population	Sex	Age	Route	Timing of Intervention	Intervention	Outcomes
Preiser 2000 (S118)	Mixed ICU (n=51)	M: 24 F: 13	Enriched group: 57±3 Control group: 57±4	EN	From day 1-7	<p><b>a: Enriched group:</b> Antioxidant rich formula via EN (133 µg/100 ml vitamin A, 13.3 mg/100 ml vitamin C, and 4.94 mg/100 ml vitamin E)</p> <p><b>b: Control group:</b> Isonitrogenous, isocaloric standard formula via EN (66.7 µg/100 ml vitamin A, 5 mg/100 ml vitamin C and 0.81 mg/100 ml vitamin E)</p> <p>EN both groups</p> <p>Allocated ??/?? Assessed 20/17 (14 patients excluded after randomization: n=37)</p>	<p>Follow-up: 28 days Study period: 7 days</p> <p><u>Main study endpoints:</u></p> <ul style="list-style-type: none"> <li>• Ex vivo LDL tolerance to oxidative stress</li> </ul> <p><u>Outcomes (related to study objectives):</u></p> <ul style="list-style-type: none"> <li>• Mortality (ICU &amp; hospital – 28 days)</li> <li>• Infections</li> <li>• LICU</li> <li>• MN status (Vitamin A, β-carotene, tocopherols)</li> <li>• TBARS (MDA)</li> </ul>

Study (& Study ID)	Population	Sex	Age	Route	Timing of Intervention	Intervention	Outcomes
Rock 1997 (S124)	Burns >20% TBSA (n=27)	M: 19 F: 6 (n=25 ?)	47±5 (mean ± SEM)	EN or PO	Unable to determine exact timing of intervention [presumed from study inclusion to day 21 (reported as end of study period)]	<p><b>a: Beta-carotene group:</b> 30 mg/day beta-carotene (supplied as capsules; contents of capsule mixed in 30ml enteral formula / meal)</p> <p><b>b: Placebo group:</b> Received placebo</p> <p>Mostly EN with minimal oral intake</p> <p>Allocated: ??/?? Assessed: 12/14 (1 patient withdrawn due to difficulties with venous access: n=26)</p>	<p>Follow-up and study period: 21 days</p> <p><u>Main study endpoints:</u></p> <ul style="list-style-type: none"> <li>• Plasma AO MN concentrations</li> </ul> <p><u>Outcomes (related to study objectives):</u></p> <ul style="list-style-type: none"> <li>• No primary objectives covered</li> <li>• MN status (Plasma carotenoids, incl β-carotene, retinol, vitamin C and α-tocopherol)</li> </ul>

Study (& Study ID)	Population	Sex	Age	Route	Timing of Intervention	Intervention	Outcomes
Rümelin 2005 (S128)	Surgical post-operative ICU (elective surgeries) (n=57)	NA	Intervention group: 57±11 Control group: 58±10	IV	Substitution on the 2 <sup>nd</sup> and 3 <sup>rd</sup> postoperative days	<p><b>a: Intervention group:</b> Substitution on the 2<sup>nd</sup> &amp; 3<sup>rd</sup> postoperative days in dependence on the actual AA concentration on a day-to-day basis. AA IV up to four times within 12 h depending upon the initial AA concentration [<math>&lt;34.1 \mu\text{mol/l}</math> (4x500 mg AA); <math>\leq 56.8 \mu\text{mol/l}</math> (2x500 mg AA); <math>\leq 68.2 \mu\text{mol/l}</math> (1x500 mg AA); <math>&gt;68.2 \mu\text{mol/l}</math> (no substitution)]. AA was given IV in 100 ml 5% glucose for 30 min.</p> <p><b>b: Control group:</b> Control group patients were not substituted</p> <p>No further information re nutrition support/care</p> <p>Allocated: ??/?? Assessed: 28/27 (patients withdrawn: missing post-op values; n=55)</p>	<p>Follow-up and study period: 3 days</p> <p><u>Main study endpoints:</u></p> <ul style="list-style-type: none"> <li>• MN status (AA) after substitution</li> </ul> <p><u>Outcomes (related to study objectives):</u></p> <ul style="list-style-type: none"> <li>• No primary objectives covered</li> <li>• MN status (AA) after substitution</li> </ul>

Study (& Study ID)	Population	Sex	Age	Route	Timing of Intervention	Intervention	Outcomes
Siriwardena 2007 (S135)	Severe acute pancreatitis (n=43)	M: 15 F: 28	AO group: 64 (13) Placebo group: 71 (14)	IV	Day 1-7	<p><b>a: AO group:</b> IV AO regimen: <b>Se in 50 ml normal saline (dose as elemental Se)</b> D1: 1000 µg Se over 24h IV D2: 400 µg Se over 24h IV D3: 200 µg Se over 24h IV, (continued for up to day 7) <b>AA in 100 ml normal saline</b> D1: 2000 mg over 24h IV D2: 2000 mg over 24h IV D3: 1000 mg over 24h IV (continued for up to day 7) <b>n-Acetylcysteine</b> D1: 300 mg/kg over 24h IV D2: 150 mg/kg over 24h IV D3: 75 mg/kg over 24h IV (continued for up to day 7) <b>b: Placebo group:</b> IV placebo for 7 days</p> <p>If patients in either group required IV fluids for &lt; 7 days: active or placebo treatment stopped upon cessation of IV fluid replacement</p> <p>No specific detail re nutrition support/care; states maximal conventional treatment for all</p> <p>Allocated: 22/21 Assessed: 22/21</p>	<p>Follow-up: discharge or death</p> <p><u>Main study endpoints:</u></p> <ul style="list-style-type: none"> <li>• Organ dysfunction</li> </ul> <p><u>Outcomes (related to study objectives):</u></p> <ul style="list-style-type: none"> <li>• Mortality</li> <li>• LICU</li> <li>• LOS</li> <li>• Morbidity (APACHE II)</li> <li>• MN status (AA, Se)</li> <li>• CRP</li> <li>• GSH/GSSG ratio</li> </ul>

Study (& Study ID)	Population	Sex	Age	Route	Timing of Intervention	Intervention	Outcomes
Young 1996 (S146)	Severely head-injured patients, ventilated (n=68)	M: 55 F: 13	Zn-supplemented: 34.6±14 Zn-standard: 35.9±15 (range 18-65)	IV then PO	From day 1-15 (PN), then orally till 3 months after injury	<p><b>a: Zn-supplemented group:</b> 12 mg elemental zinc via PN (0-15 days), then progressing to oral zinc (168 mg zinc gluconate, 22 mg elemental zinc) till 3 months after injury</p> <p><b>b: Zn-standard group:</b> 2.5 mg elemental zinc via PN (0-15 days), then progressing to oral placebo till 3 months after injury</p> <p>TPN in both groups; weaned to the enteral route as tolerated</p> <p>Allocated 33/35 Assessed 33/35</p>	<p>Follow-up: 28 days Study period: 7 days</p> <p><u>Main study endpoints:</u></p> <ul style="list-style-type: none"> <li>Neurological outcome (GCS: 28 days)</li> </ul> <p><u>Outcomes (related to study objectives):</u></p> <ul style="list-style-type: none"> <li>Mortality</li> <li>MN status (Zn)</li> </ul>

\*Indicates the major publication for the study

α-T: α-Tocopherol; AA: Ascorbic acid; AO: Antioxidant; AOE: Antioxidant enzymes; AOX: Antioxidant; BSA: Body surface area; CRP: C-reactive protein; Cu: Copper; D: Day; D5W: 5% dextrose in water; E: Erythrocyte; EN: Enteral nutrition; GCS: Glasgow coma scale; GPx-3: Glutathione peroxidase-3; GR: Glutathione reductase; GSH: Glutathione; GSH/GSSG ratio: glutathione/glutathione disulphide ratio; GSHPx: Selenoenzyme glutathione peroxidase; ICU: Intensive care unit; IV: Intravenous; LDL: Low density lipoprotein; LICU: Length of ICU stay; LOS: Length of hospital stay; MDA: Malondialdehyde acid; MN: Micronutrient; NA: Not available; P: Plasma; PN: Parenteral nutrition; PO: Per os; RRT: RBC: Red blood cell; Renal replacement therapy; SBU: Standard Burned Unit; SD: Standard deviation; Se: Selenium; SIRS: Systemic inflammatory response syndrome; SOD: Superoxide dismutase; TAC: Total antioxidant capacity; TAS: Total antioxidant status; TBARS: Thiobarbituric acid-reacting substances; TE: Trace element; WBC: White blood cell; Zn: Zinc

**Appendix 6.7: Assessment of methodological quality of studies**

Study: **Angstwurm 2007 (S3)**; Multiple Centre Trial (MCT) / Prospective, Randomized, Placebo-controlled trial

Criteria used to assess methodological quality <sup>152</sup>				Cochrane “Risk of bias” assessment tool <sup>11</sup>		Comments (additional)
Criteria	Score			Comments (additional)		
	0	1	2			
<b>Randomization</b>	...	Not concealed or not sure	Concealed randomization	Not described	<b>Sequence Generation</b>	
<b>Blinding</b>	Not blinded	...	Adjudicators blinded	Double-blind	Yes (low risk of bias) <input checked="" type="checkbox"/>	Computer programme used
<b>Analysis</b>	Other	...	Intention to treat	ITT: No	No (high risk of bias) <input type="checkbox"/>	
<b>Patient selection</b>	Selected patients or unable to tell	Consecutive eligible patients	...	Unable to tell	Unclear (uncertain risk of bias) <input type="checkbox"/>	
<b>Comparability of groups at baseline</b>	No or not sure	Yes	...	Significant differences present	<b>Allocation Concealment</b>	
<b>Extent of follow-up</b>	<100%	100%	...		Yes (low risk of bias) <input type="checkbox"/>	“As-treated” analysis done (not ITT analysis)
<b>Treatment protocol</b>	Poorly described	Reproducibly described	...		No (high risk of bias) <input checked="" type="checkbox"/>	
<b>Co-interventions</b>	Not described	Described but not equal or not sure	Well described and all equal		Unclear (uncertain risk of bias) <input type="checkbox"/>	
<b>Outcomes</b>	Not described	Partially described	Objectively defined		<b>Blinding</b>	
<b>Score: 9/14</b>					Yes (low risk of bias) <input checked="" type="checkbox"/>	
					No (high risk of bias) <input type="checkbox"/>	
					Unclear (uncertain risk of bias) <input checked="" type="checkbox"/>	
					<b>Incomplete Outcome Data</b>	
					Yes (low risk of bias) <input type="checkbox"/>	“As-treated” analysis done (not ITT analysis)
					No (high risk of bias) <input checked="" type="checkbox"/>	
					Unclear (uncertain risk of bias) <input type="checkbox"/>	
					<b>Selective Outcome Reporting</b>	
					Yes (low risk of bias) <input checked="" type="checkbox"/>	
					No (high risk of bias) <input type="checkbox"/>	
					Unclear (uncertain risk of bias) <input type="checkbox"/>	
					<b>Other Potential Threats to Validity</b>	
					Yes (low risk of bias) <input type="checkbox"/>	Significant differences in baseline characteristics
					No (high risk of bias) <input type="checkbox"/>	
					Unclear (uncertain risk of bias) <input checked="" type="checkbox"/>	

Study: **Angstwurm 2004 & 1999\* (S5 & S6\*)**; Single Centre Trial (SCT) / Controlled, Randomized, Prospective, Open Label, Pilot study

Criteria used to assess methodological quality <sup>152</sup>				Cochrane "Risk of bias" assessment tool <sup>11</sup>	
Criteria	Score			Comments (additional)	Comments (additional)
	0	1	2		
<b>Randomization</b>	...	Not concealed or not sure	Concealed randomization	Randomization not described	Not described
<b>Blinding</b>	Not blinded	...	Adjudicators blinded	Open label	
<b>Analysis</b>	Other	...	Intention to treat		
<b>Patient selection</b>	Selected patients or unable to tell	Consecutive eligible patients	...		
<b>Comparability of groups at baseline</b>	No or not sure	Yes	...		
<b>Extent of follow-up</b>	<100%	100%	...		
<b>Treatment protocol</b>	Poorly described	Reproducibly described	...		
<b>Co-interventions</b>	Not described	Described but not equal or not sure	Well described and all equal		
<b>Outcomes</b>	Not described	Partially described	Objectively defined		
<b>Score: 11/14</b>					

Cochrane "Risk of bias" assessment tool <sup>11</sup>		Comments (additional)
<b>Sequence Generation</b>		
Yes (low risk of bias)	<input type="checkbox"/>	Not described
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input checked="" type="checkbox"/>	
<b>Allocation Concealment</b>		
Yes (low risk of bias)	<input type="checkbox"/>	Randomization not described
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input checked="" type="checkbox"/>	
<b>Blinding</b>		
Yes (low risk of bias)	<input type="checkbox"/>	
No (high risk of bias)	<input checked="" type="checkbox"/>	
Unclear (uncertain risk of bias)	<input type="checkbox"/>	
<b>Incomplete Outcome Data</b>		
Yes (low risk of bias)	<input checked="" type="checkbox"/>	
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input type="checkbox"/>	
<b>Selective Outcome Reporting</b>		
Yes (low risk of bias)	<input checked="" type="checkbox"/>	
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input type="checkbox"/>	
<b>Other Potential Threats to Validity</b>		
Yes (low risk of bias)	<input checked="" type="checkbox"/>	
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input type="checkbox"/>	

Study: **Berger 2001 & 2001 (S14\* & S26)**; Single Centre Trial (SCT) / Prospective, Double-blind, Randomized, Supplementation trial

Criteria used to assess methodological quality <sup>152</sup>				Cochrane “Risk of bias” assessment tool <sup>11</sup>	
Criteria	Score			Comments (additional)	Comments (additional)
	0	1	2		
<b>Randomization</b>	...	Not concealed or not sure	Concealed randomization		
<b>Blinding</b>	Not blinded	...	Adjudicators blinded	Double-blind	
<b>Analysis</b>	Other	...	Intention to treat		
<b>Patient selection</b>	Selected patients or unable to tell	Consecutive eligible patients	...	Not described	
<b>Comparability of groups at baseline</b>	No or not sure	Yes	...	Significant difference in sex ratio	
<b>Extent of follow-up</b>	<100%	100%	...		
<b>Treatment protocol</b>	Poorly described	Reproducibly described	...		
<b>Co-interventions</b>	Not described	Described but not equal or not sure	Well described and all equal		
<b>Outcomes</b>	Not described	Partially described	Objectively defined		
<b>Score: 12/14</b>					

Cochrane “Risk of bias” assessment tool <sup>11</sup>		Comments (additional)
<b>Sequence Generation</b>		
Yes (low risk of bias)	<input type="checkbox"/>	
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input checked="" type="checkbox"/>	
<b>Allocation Concealment</b>		
Yes (low risk of bias)	<input checked="" type="checkbox"/>	
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input type="checkbox"/>	
<b>Blinding</b>		
Yes (low risk of bias)	<input checked="" type="checkbox"/>	
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input type="checkbox"/>	
<b>Incomplete Outcome Data</b>		
Yes (low risk of bias)	<input checked="" type="checkbox"/>	
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input type="checkbox"/>	
<b>Selective Outcome Reporting</b>		
Yes (low risk of bias)	<input checked="" type="checkbox"/>	
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input type="checkbox"/>	
<b>Other Potential Threats to Validity</b>		
Yes (low risk of bias)	<input type="checkbox"/>	
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input checked="" type="checkbox"/>	Potential bias: significant difference in sex ratio

Study: **Berger 2007 (S15\* & S18)**; Single Centre Trial (SCT) / Prospective, Randomized, Placebo-controlled trial

Criteria used to assess methodological quality <sup>152</sup>					Cochrane "Risk of bias" assessment tool <sup>11</sup>			
Criteria	Score			Comments (additional)	Comments (additional)			
	0	1	2		Sequence Generation	Allocation Concealment	Blinding	
Randomization	...	Not concealed or not sure	Concealed randomization	Not described	Yes (low risk of bias) <input type="checkbox"/>	No (high risk of bias) <input type="checkbox"/>	Unclear (uncertain risk of bias) <input checked="" type="checkbox"/>	
Blinding	Not blinded	...	Adjudicators blinded	Not stated?	Yes (low risk of bias) <input type="checkbox"/>	No (high risk of bias) <input type="checkbox"/>	Unclear (uncertain risk of bias) <input checked="" type="checkbox"/>	
Analysis	Other	...	Intention to treat		<b>Blinding</b>			
Patient selection	Selected patients or unable to tell	Consecutive eligible patients	...	Unable to tell	Yes (low risk of bias) <input type="checkbox"/>	No (high risk of bias) <input type="checkbox"/>	Unclear (uncertain risk of bias) <input checked="" type="checkbox"/>	Not clear
Comparability of groups at baseline	No or not sure	Yes	...		<b>Incomplete Outcome Data</b>			
Extent of follow-up	<100%	100%	...		Yes (low risk of bias) <input checked="" type="checkbox"/>	No (high risk of bias) <input type="checkbox"/>	Unclear (uncertain risk of bias) <input type="checkbox"/>	
Treatment protocol	Poorly described	Reproducibly described	...		<b>Selective Outcome Reporting</b>			
Co-interventions	Not described	Described but not equal or not sure	Well described and all equal		Yes (low risk of bias) <input type="checkbox"/>	No (high risk of bias) <input checked="" type="checkbox"/>	Unclear (uncertain risk of bias) <input type="checkbox"/>	Hospital stay mentioned (NS) but no data provided
Outcomes	Not described	Partially described	Objectively defined	Hospital stay mentioned (NS) but no data provided	<b>Other Potential Threats to Validity</b>			
					Yes (low risk of bias) <input checked="" type="checkbox"/>	No (high risk of bias) <input type="checkbox"/>	Unclear (uncertain risk of bias) <input type="checkbox"/>	

**Score: 9/14**

Study: **Berger 1998 (S33)**; Single Centre Trial (SCT) / Prospective, Randomized, Placebo-controlled trial

Criteria used to assess methodological quality <sup>152</sup>					Cochrane “Risk of bias” assessment tool <sup>11</sup>		
Criteria	Score			Comments (additional)	Comments (additional)		
	0	1	2				
<b>Randomization</b>	...	Not concealed or not sure	Concealed randomization		<b>Sequence Generation</b>		
<b>Blinding</b>	Not blinded	...	Adjudicators blinded	Double-blind	Yes (low risk of bias)	<input type="checkbox"/>	
<b>Analysis</b>	Other	...	Intention to treat		No (high risk of bias)	<input type="checkbox"/>	
<b>Patient selection</b>	Selected patients or unable to tell	Consecutive eligible patients	...	Unable to tell	Unclear (uncertain risk of bias)	<input checked="" type="checkbox"/>	
<b>Comparability of groups at baseline</b>	No or not sure	Yes	...		<b>Allocation Concealment</b>		
<b>Extent of follow-up</b>	<100%	100%	...		Yes (low risk of bias)	<input checked="" type="checkbox"/>	
<b>Treatment protocol</b>	Poorly described	Reproducibly described	...		No (high risk of bias)	<input type="checkbox"/>	
<b>Co-interventions</b>	Not described	Described but not equal or not sure	Well described and all equal	Partially described: nutritional management	Unclear (uncertain risk of bias)	<input type="checkbox"/>	
<b>Outcomes</b>	Not described	Partially described	Objectively defined		<b>Blinding</b>		
<b>Score: 12/14</b>					Yes (low risk of bias)	<input checked="" type="checkbox"/>	
					No (high risk of bias)	<input type="checkbox"/>	
					Unclear (uncertain risk of bias)	<input type="checkbox"/>	
					<b>Incomplete Outcome Data</b>		
					Yes (low risk of bias)	<input checked="" type="checkbox"/>	
					No (high risk of bias)	<input type="checkbox"/>	
					Unclear (uncertain risk of bias)	<input type="checkbox"/>	
					<b>Selective Outcome Reporting</b>		
					Yes (low risk of bias)	<input checked="" type="checkbox"/>	
					No (high risk of bias)	<input type="checkbox"/>	
					Unclear (uncertain risk of bias)	<input type="checkbox"/>	
					<b>Other Potential Threats to Validity</b>		
					Yes (low risk of bias)	<input checked="" type="checkbox"/>	
					No (high risk of bias)	<input type="checkbox"/>	
					Unclear (uncertain risk of bias)	<input type="checkbox"/>	

Study: **Cheng 2006 (S52)**; Single Centre Trial (SCT) / Randomized, Single-blind, Intervention study

Criteria used to assess methodological quality <sup>152</sup>				Cochrane "Risk of bias" assessment tool <sup>11</sup>	
Criteria	Score			Comments	Comments
	0	1	2		
Randomization	...	Not concealed or not sure	Concealed randomization	Not described	
Blinding	Not blinded	...	Adjudicators blinded	Single-blind	
Analysis	Other	...	Intention to treat		
Patient selection	Selected patients or unable to tell	Consecutive eligible patients	...	Unable to tell	
Comparability of groups at baseline	No or not sure	Yes	...	Significant differences in height and BMI	
Extent of follow-up	<100%	100%	...		
Treatment protocol	Poorly described	Reproducibly described	...		
Co-interventions	Not described	Described but not equal or not sure	Well described and all equal	Partially described: nutritional management	
Outcomes	Not described	Partially described	Objectively defined		
<b>Score: 10/14</b>					

Cochrane "Risk of bias" assessment tool <sup>11</sup>		Comments
<b>Sequence Generation</b>		
Yes (low risk of bias)	<input type="checkbox"/>	
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input type="checkbox"/>	
<b>Allocation Concealment</b>		
Yes (low risk of bias)	<input type="checkbox"/>	
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input type="checkbox"/>	
<b>Blinding</b>		
Yes (low risk of bias)	<input type="checkbox"/>	
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input type="checkbox"/>	
<b>Incomplete Outcome Data</b>		
Yes (low risk of bias)	<input type="checkbox"/>	
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input type="checkbox"/>	
<b>Selective Outcome Reporting</b>		
Yes (low risk of bias)	<input type="checkbox"/>	
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input type="checkbox"/>	
<b>Other Potential Threats to Validity</b>		
Yes (low risk of bias)	<input type="checkbox"/>	
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input type="checkbox"/>	Significant differences in baseline data; no placebo used

Study: **Crimi 2004 (S54)**; Multiple Centre Trial (MCT) / Randomized, Double-blind, Placebo-controlled, Supplementation trial

Criteria used to assess methodological quality <sup>152</sup>					Cochrane “Risk of bias” assessment tool <sup>11</sup>		
Criteria	Score			Comments (additional)	Comments (additional)		
	0	1	2				
<b>Randomization</b>	...	Not concealed or not sure	Concealed randomization	By pharmacy	<b>Sequence Generation</b>	Yes (low risk of bias) <input checked="" type="checkbox"/>	Computer-generated random numbers
<b>Blinding</b>	Not blinded	...	Adjudicators blinded	Double-blind	No (high risk of bias) <input type="checkbox"/>	Unclear (uncertain risk of bias) <input type="checkbox"/>	
<b>Analysis</b>	Other	...	Intention to treat	ITT analysis for primary objectives	<b>Allocation Concealment</b>	Yes (low risk of bias) <input checked="" type="checkbox"/>	
<b>Patient selection</b>	Selected patients or unable to tell	Consecutive eligible patients	...		No (high risk of bias) <input type="checkbox"/>	Unclear (uncertain risk of bias) <input type="checkbox"/>	
<b>Comparability of groups at baseline</b>	No or not sure	Yes	...		<b>Blinding</b>	Yes (low risk of bias) <input checked="" type="checkbox"/>	
<b>Extent of follow-up</b>	<100%	100%	...		No (high risk of bias) <input type="checkbox"/>	Unclear (uncertain risk of bias) <input type="checkbox"/>	
<b>Treatment protocol</b>	Poorly described	Reproducibly described	...		<b>Incomplete Outcome Data</b>	Yes (low risk of bias) <input checked="" type="checkbox"/>	
<b>Co-interventions</b>	Not described	Described but not equal or not sure	Well described and all equal		No (high risk of bias) <input type="checkbox"/>	Unclear (uncertain risk of bias) <input type="checkbox"/>	Infections stated to be NS different; no data provided
<b>Outcomes</b>	Not described	Partially described	Objectively defined	Infections stated to be NS different; no data provided	<b>Selective Outcome Reporting</b>	Yes (low risk of bias) <input type="checkbox"/>	
<b>Score: 13/14</b>					No (high risk of bias) <input checked="" type="checkbox"/>	Unclear (uncertain risk of bias) <input type="checkbox"/>	
					<b>Other Potential Threats to Validity</b>	Yes (low risk of bias) <input checked="" type="checkbox"/>	
					No (high risk of bias) <input type="checkbox"/>	Unclear (uncertain risk of bias) <input type="checkbox"/>	

Study: **Forceville 2007 (S65)**; Multiple Centre Trial (MCT) / Prospective, Placebo-controlled, Randomized, Double-blind study

Criteria used to assess methodological quality <sup>152</sup>				Cochrane "Risk of bias" assessment tool <sup>11</sup>	
Criteria	Score			Comments (additional)	Comments (additional)
	0	1	2		
Randomization	...	Not concealed or not sure	Concealed randomization	Sequentially numbered boxes delivered to hospitals	Not described
Blinding	Not blinded	...	Adjudicators blinded	Double-blind	Sequentially numbered boxes
Analysis	Other	...	Intention to treat		
Patient selection	Selected patients or unable to tell	Consecutive eligible patients	...	Unable to tell	
Comparability of groups at baseline	No or not sure	Yes	...	Se group: significant differences: admission category	
Extent of follow-up	<100%	100%	...		
Treatment protocol	Poorly described	Reproducibly described	...		
Co-interventions	Not described	Described but not equal or not sure	Well described and all equal		
Outcomes	Not described	Partially described	Objectively defined		

**Score: 10/14**

Cochrane "Risk of bias" assessment tool <sup>11</sup>		Comments (additional)
<b>Sequence Generation</b>		
Yes (low risk of bias)	<input type="checkbox"/>	Not described
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input checked="" type="checkbox"/>	
<b>Allocation Concealment</b>		
Yes (low risk of bias)	<input checked="" type="checkbox"/>	Sequentially numbered boxes
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input type="checkbox"/>	
<b>Blinding</b>		
Yes (low risk of bias)	<input checked="" type="checkbox"/>	
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input type="checkbox"/>	
<b>Incomplete Outcome Data</b>		
Yes (low risk of bias)	<input checked="" type="checkbox"/>	
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input type="checkbox"/>	
<b>Selective Outcome Reporting</b>		
Yes (low risk of bias)	<input checked="" type="checkbox"/>	
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input type="checkbox"/>	
<b>Other Potential Threats to Validity</b>		
Yes (low risk of bias)	<input type="checkbox"/>	Baseline data significant differences: risk of bias
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input checked="" type="checkbox"/>	

Study: **Galley 1997 (S68)**; Single Centre Trial (SCT) / Randomized, Controlled trial

Criteria used to assess methodological quality <sup>152</sup>				Cochrane “Risk of bias” assessment tool <sup>11</sup>	
Criteria	Score			Comments (additional)	Comments (additional)
	0	1	2		
<b>Randomization</b>	...	Not concealed or not sure	Concealed randomization	Not described	<input type="checkbox"/>
<b>Blinding</b>	Not blinded	...	Adjudicators blinded		
<b>Analysis</b>	Other	...	Intention to treat		<input checked="" type="checkbox"/>
<b>Patient selection</b>	Selected patients or unable to tell	Consecutive eligible patients	...		<input type="checkbox"/>
<b>Comparability of groups at baseline</b>	No or not sure	Yes	...	Unable to tell	<input checked="" type="checkbox"/>
<b>Extent of follow-up</b>	<100%	100%	...		<input type="checkbox"/>
<b>Treatment protocol</b>	Poorly described	Reproducibly described	...		<input type="checkbox"/>
<b>Co-interventions</b>	Not described	Described but not equal or not sure	Well described and all equal		<input type="checkbox"/>
<b>Outcomes</b>	Not described	Partially described	Objectively defined		<input type="checkbox"/>
<b>Score: 8/14</b>					

Study: **Maderazo 1991 (S92)**; Single Centre Trial (SCT) / Prospective, Placebo-controlled, Double-blind, Randomized study

Criteria used to assess methodological quality <sup>152</sup>					Cochrane "Risk of bias" assessment tool <sup>11</sup>	
Criteria	Score			Comments (additional)		
	0	1	2			Comments (additional)
<b>Randomization</b>	...	Not concealed or not sure	Concealed randomization	Randomized block design	<b>Sequence Generation</b> Yes (low risk of bias) <input type="checkbox"/> No (high risk of bias) <input type="checkbox"/> Unclear (uncertain risk of bias) <input checked="" type="checkbox"/>	
<b>Blinding</b>	Not blinded	...	Adjudicators blinded	Double-blind	<b>Allocation Concealment</b> Yes (low risk of bias) <input checked="" type="checkbox"/> No (high risk of bias) <input type="checkbox"/> Unclear (uncertain risk of bias) <input type="checkbox"/>	
<b>Analysis</b>	Other	...	Intention to treat		<b>Blinding</b> Yes (low risk of bias) <input checked="" type="checkbox"/> No (high risk of bias) <input type="checkbox"/> Unclear (uncertain risk of bias) <input type="checkbox"/>	
<b>Patient selection</b>	Selected patients or unable to tell	Consecutive eligible patients	...	Unable to tell	<b>Incomplete Outcome Data</b> Yes (low risk of bias) <input checked="" type="checkbox"/> No (high risk of bias) <input type="checkbox"/> Unclear (uncertain risk of bias) <input type="checkbox"/>	
<b>Comparability of groups at baseline</b>	No or not sure	Yes	...	Significant differences not discussed; no p-values provided	<b>Selective Outcome Reporting</b> Yes (low risk of bias) <input checked="" type="checkbox"/> No (high risk of bias) <input type="checkbox"/> Unclear (uncertain risk of bias) <input type="checkbox"/>	
<b>Extent of follow-up</b>	<100%	100%	...		<b>Other Potential Threats to Validity</b> Yes (low risk of bias) <input type="checkbox"/> No (high risk of bias) <input type="checkbox"/> Unclear (uncertain risk of bias) <input checked="" type="checkbox"/>	Not known if baseline data has any significant differences: unclear if risk of bias exists
<b>Treatment protocol</b>	Poorly described	Reproducibly described	...			
<b>Co-interventions</b>	Not described	Described but not equal or not sure	Well described and all equal			
<b>Outcomes</b>	Not described	Partially described	Objectively defined			

**Score: 9/14**

Study: **Mishra 2007 (S103)**; Single Centre Trial (SCT) / Prospective, Randomized study

Criteria used to assess methodological quality <sup>152</sup>				Cochrane “Risk of bias” assessment tool <sup>11</sup>	
Criteria	Score			Comments (additional)	Comments (additional)
	0	1	2		
<b>Randomization</b>	...	Not concealed or not sure	Concealed randomization	Not described	
<b>Blinding</b>	Not blinded	...	Adjudicators blinded	Double-blind	
<b>Analysis</b>	Other	...	Intention to treat		
<b>Patient selection</b>	Selected patients or unable to tell	Consecutive eligible patients	...	Unable to tell	
<b>Comparability of groups at baseline</b>	No or not sure	Yes	...	Not discussed but p-values given	
<b>Extent of follow-up</b>	<100%	100%	...		
<b>Treatment protocol</b>	Poorly described	Reproducibly described	...		
<b>Co-interventions</b>	Not described	Described but not equal or not sure	Well described and all equal		
<b>Outcomes</b>	Not described	Partially described	Objectively defined		
<b>Score: 12/14</b>					

Cochrane “Risk of bias” assessment tool <sup>11</sup>		Comments (additional)
<b>Sequence Generation</b>		
Yes (low risk of bias)	<input type="checkbox"/>	
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input checked="" type="checkbox"/>	
<b>Allocation Concealment</b>		
Yes (low risk of bias)	<input type="checkbox"/>	
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input checked="" type="checkbox"/>	
<b>Blinding</b>		
Yes (low risk of bias)	<input checked="" type="checkbox"/>	
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input type="checkbox"/>	
<b>Incomplete Outcome Data</b>		
Yes (low risk of bias)	<input checked="" type="checkbox"/>	
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input type="checkbox"/>	
<b>Selective Outcome Reporting</b>		
Yes (low risk of bias)	<input checked="" type="checkbox"/>	
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input type="checkbox"/>	
<b>Other Potential Threats to Validity</b>		
Yes (low risk of bias)	<input type="checkbox"/>	
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input checked="" type="checkbox"/>	Not placebo controlled / Standard treatment

Study: **Nathens 2002 (S107)**; Single Centre Trial (SCT) / Randomized, Prospective study

Criteria used to assess methodological quality <sup>152</sup>					Cochrane "Risk of bias" assessment tool <sup>11</sup>		
Criteria	Score			Comments (additional)	Comments (additional)		
	0	1	2				
<b>Randomization</b>	...	Not concealed or not sure	Concealed randomization		<b>Sequence Generation</b>		
<b>Blinding</b>	Not blinded	...	Adjudicators blinded		Yes (low risk of bias) <input checked="" type="checkbox"/>		Computer generated random number sequence
<b>Analysis</b>	Other	...	Intention to treat		No (high risk of bias) <input type="checkbox"/>		
<b>Patient selection</b>	Selected patients or unable to tell	Consecutive eligible patients	...	Unable to tell	Unclear (uncertain risk of bias) <input type="checkbox"/>		
<b>Comparability of groups at baseline</b>	No or not sure	Yes	...		<b>Allocation Concealment</b>		
<b>Extent of follow-up</b>	<100%	100%	...		Yes (low risk of bias) <input type="checkbox"/>		Pharmacy
<b>Treatment protocol</b>	Poorly described	Reproducibly described	...		No (high risk of bias) <input type="checkbox"/>		
<b>Co-interventions</b>	Not described	Described but not equal or not sure	Well described and all equal	Not sure if co-interventions were equal	Unclear (uncertain risk of bias) <input checked="" type="checkbox"/>		
<b>Outcomes</b>	Not described	Partially described	Objectively defined		<b>Blinding</b>		
<b>Score: 7/14</b>					Yes (low risk of bias) <input type="checkbox"/>		
					No (high risk of bias) <input checked="" type="checkbox"/>		No ITT analysis: "as-treated" analysis done
					Unclear (uncertain risk of bias) <input type="checkbox"/>		
					<b>Incomplete Outcome Data</b>		
					Yes (low risk of bias) <input type="checkbox"/>		
					No (high risk of bias) <input checked="" type="checkbox"/>		
					Unclear (uncertain risk of bias) <input type="checkbox"/>		
					<b>Selective Outcome Reporting</b>		
					Yes (low risk of bias) <input checked="" type="checkbox"/>		
					No (high risk of bias) <input type="checkbox"/>		
					Unclear (uncertain risk of bias) <input type="checkbox"/>		
					<b>Other Potential Threats to Validity</b>		
					Yes (low risk of bias) <input type="checkbox"/>		Not placebo controlled
					No (high risk of bias) <input type="checkbox"/>		
					Unclear (uncertain risk of bias) <input checked="" type="checkbox"/>		

Study: **Porter 1999 (S115)**; Single Centre Trial (SCT) / Prospective, Randomized trial

Criteria used to assess methodological quality <sup>152</sup>					Cochrane “Risk of bias” assessment tool <sup>11</sup>		
Criteria	Score			Comments (additional)	Comments (additional)		
	0	1	2				
<b>Randomization</b>	...	Not concealed or not sure	Concealed randomization	Sealed manilla envelopes	<b>Sequence Generation</b>		
<b>Blinding</b>	Not blinded	...	Adjudicators blinded		Yes (low risk of bias)	<input type="checkbox"/>	
<b>Analysis</b>	Other	...	Intention to treat		No (high risk of bias)	<input type="checkbox"/>	
<b>Patient selection</b>	Selected patients or unable to tell	Consecutive eligible patients	...	Unable to tell	Unclear (uncertain risk of bias)	<input checked="" type="checkbox"/>	
<b>Comparability of groups at baseline</b>	No or not sure	Yes	...		<b>Allocation Concealment</b>		
<b>Extent of follow-up</b>	<100%	100%	...		Yes (low risk of bias)	<input checked="" type="checkbox"/>	
<b>Treatment protocol</b>	Poorly described	Reproducibly described	...		No (high risk of bias)	<input type="checkbox"/>	
<b>Co-interventions</b>	Not described	Described but not equal or not sure	Well described and all equal		Unclear (uncertain risk of bias)	<input type="checkbox"/>	
<b>Outcomes</b>	Not described	Partially described	Objectively defined		<b>Blinding</b>		
<b>Score: 9/14</b>					Yes (low risk of bias)	<input type="checkbox"/>	
					No (high risk of bias)	<input checked="" type="checkbox"/>	
					Unclear (uncertain risk of bias)	<input type="checkbox"/>	
					<b>Incomplete Outcome Data</b>		
					Yes (low risk of bias)	<input checked="" type="checkbox"/>	
					No (high risk of bias)	<input type="checkbox"/>	
					Unclear (uncertain risk of bias)	<input type="checkbox"/>	
					<b>Selective Outcome Reporting</b>		
					Yes (low risk of bias)	<input checked="" type="checkbox"/>	
					No (high risk of bias)	<input type="checkbox"/>	
					Unclear (uncertain risk of bias)	<input type="checkbox"/>	
					<b>Other Potential Threats to Validity</b>		
					Yes (low risk of bias)	<input type="checkbox"/>	
					No (high risk of bias)	<input type="checkbox"/>	
					Unclear (uncertain risk of bias)	<input checked="" type="checkbox"/>	
							Not placebo controlled

Study: **Preiser 2000 (S118)**; Single Centre Trial (SCT) / Prospective, Randomized, Double-blind, Placebo-controlled study

Criteria used to assess methodological quality <sup>152</sup>				Cochrane “Risk of bias” assessment tool <sup>11</sup>	
Criteria	Score			Comments (additional)	Comments (additional)
	0	1	2		
Randomization	...	Not concealed or not sure	Concealed randomization		
Blinding	Not blinded	...	Adjudicators blinded	Double-blind	
Analysis	Other	...	Intention to treat		
Patient selection	Selected patients or unable to tell	Consecutive eligible patients	...	Unable to tell	
Comparability of groups at baseline	No or not sure	Yes	...		
Extent of follow-up	<100%	100%	...		
Treatment protocol	Poorly described	Reproducibly described	...		
Co-interventions	Not described	Described but not equal or not sure	Well described and all equal		
Outcomes	Not described	Partially described	Objectively defined		
<b>Score: 8/14</b>					

Cochrane “Risk of bias” assessment tool <sup>11</sup>		Comments (additional)
<b>Sequence Generation</b>		
Yes (low risk of bias)	<input checked="" type="checkbox"/>	Computerized random number table
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input type="checkbox"/>	
<b>Allocation Concealment</b>		
Yes (low risk of bias)	<input type="checkbox"/>	Not sure
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input checked="" type="checkbox"/>	
<b>Blinding</b>		
Yes (low risk of bias)	<input checked="" type="checkbox"/>	
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input type="checkbox"/>	
<b>Incomplete Outcome Data</b>		
Yes (low risk of bias)	<input type="checkbox"/>	No ITT analysis; 14 patients excluded after randomization
No (high risk of bias)	<input checked="" type="checkbox"/>	
Unclear (uncertain risk of bias)	<input type="checkbox"/>	
<b>Selective Outcome Reporting</b>		
Yes (low risk of bias)	<input checked="" type="checkbox"/>	
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input type="checkbox"/>	
<b>Other Potential Threats to Validity</b>		
Yes (low risk of bias)	<input checked="" type="checkbox"/>	
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input type="checkbox"/>	

Study: **Rock 1997 (S124)**; Single Centre Trial (SCT) / Randomized, Controlled trial

Criteria used to assess methodological quality <sup>152</sup>				Cochrane “Risk of bias” assessment tool <sup>11</sup>	
Criteria	Score			Comments (additional)	Comments (additional)
	0	1	2		
<b>Randomization</b>	...	Not concealed or not sure	Concealed randomization	Not described	
<b>Blinding</b>	Not blinded	...	Adjudicators blinded		
<b>Analysis</b>	Other	...	Intention to treat	1 patient dropped out and not analyzed	
<b>Patient selection</b>	Selected patients or unable to tell	Consecutive eligible patients	...		
<b>Comparability of groups at baseline</b>	No or not sure	Yes	...		
<b>Extent of follow-up</b>	<100%	100%	...		
<b>Treatment protocol</b>	Poorly described	Reproducibly described	...		
<b>Co-interventions</b>	Not described	Described but not equal or not sure	Well described and all equal		
<b>Outcomes</b>	Not described	Partially described	Objectively defined		
<b>Score: 9/14</b>					

Cochrane “Risk of bias” assessment tool <sup>11</sup>		Comments (additional)
<b>Sequence Generation</b>		
Yes (low risk of bias)	<input type="checkbox"/>	
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input checked="" type="checkbox"/>	
<b>Allocation Concealment</b>		
Yes (low risk of bias)	<input type="checkbox"/>	
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input checked="" type="checkbox"/>	
<b>Blinding</b>		
Yes (low risk of bias)	<input type="checkbox"/>	
No (high risk of bias)	<input checked="" type="checkbox"/>	
Unclear (uncertain risk of bias)	<input type="checkbox"/>	
<b>Incomplete Outcome Data</b>		
Yes (low risk of bias)	<input type="checkbox"/>	
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input checked="" type="checkbox"/>	1 patient dropped out and not analyzed
<b>Selective Outcome Reporting</b>		
Yes (low risk of bias)	<input checked="" type="checkbox"/>	
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input type="checkbox"/>	
<b>Other Potential Threats to Validity</b>		
Yes (low risk of bias)	<input checked="" type="checkbox"/>	
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input type="checkbox"/>	

Study: **Rümelin 2005 (S128)**; Single Centre Trial (SCT) / Randomized, Controlled trial

Criteria used to assess methodological quality <sup>152</sup>				Cochrane “Risk of bias” assessment tool <sup>11</sup>	
Criteria	Score			Comments (additional)	Comments (additional)
	0	1	2		
<b>Randomization</b>	...	Not concealed or not sure	Concealed randomization	Not described	Not described
<b>Blinding</b>	Not blinded	...	Adjudicators blinded		
<b>Analysis</b>	Other	...	Intention to treat		
<b>Patient selection</b>	Selected patients or unable to tell	Consecutive eligible patients	...	Unable to tell	
<b>Comparability of groups at baseline</b>	No or not sure	Yes	...	Not sure if significant differences present	No ITT analysis: patients withdrawn & missing post-op values
<b>Extent of follow-up</b>	<100%	100%	...		
<b>Treatment protocol</b>	Poorly described	Reproducibly described	...		
<b>Co-interventions</b>	Not described	Described but not equal or not sure	Well described and all equal		
<b>Outcomes</b>	Not described	Partially described	Objectively defined		Not placebo controlled

**Score: 4/14**

Study: **Siriwardena 2007 (S135)**; Multiple Centre Trial (MCT) / Randomized, Double-blind, Placebo-controlled trial

Criteria used to assess methodological quality <sup>152</sup>				Cochrane "Risk of bias" assessment tool <sup>11</sup>	
Criteria	Score			Comments (additional)	Comments (additional)
	0	1	2		
<b>Randomization</b>	...	Not concealed or not sure	Concealed randomization	By pharmacy	Randomization schedule using variable length even number blocks
<b>Blinding</b>	Not blinded	...	Adjudicators blinded	Double-blind	
<b>Analysis</b>	Other	...	Intention to treat		
<b>Patient selection</b>	Selected patients or unable to tell	Consecutive eligible patients	...	Unable to tell	
<b>Comparability of groups at baseline</b>	No or not sure	Yes	...		
<b>Extent of follow-up</b>	<100%	100%	...		
<b>Treatment protocol</b>	Poorly described	Reproducibly described	...		
<b>Co-interventions</b>	Not described	Described but not equal or not sure	Well described and all equal		
<b>Outcomes</b>	Not described	Partially described	Objectively defined		
<b>Score: 13/14</b>					

<p><b>Sequence Generation</b></p> <p>Yes (low risk of bias) <input checked="" type="checkbox"/></p> <p>No (high risk of bias) <input type="checkbox"/></p> <p>Unclear (uncertain risk of bias) <input type="checkbox"/></p> <p><b>Allocation Concealment</b></p> <p>Yes (low risk of bias) <input checked="" type="checkbox"/></p> <p>No (high risk of bias) <input type="checkbox"/></p> <p>Unclear (uncertain risk of bias) <input type="checkbox"/></p> <p><b>Blinding</b></p> <p>Yes (low risk of bias) <input checked="" type="checkbox"/></p> <p>No (high risk of bias) <input type="checkbox"/></p> <p>Unclear (uncertain risk of bias) <input type="checkbox"/></p> <p><b>Incomplete Outcome Data</b></p> <p>Yes (low risk of bias) <input checked="" type="checkbox"/></p> <p>No (high risk of bias) <input type="checkbox"/></p> <p>Unclear (uncertain risk of bias) <input type="checkbox"/></p> <p><b>Selective Outcome Reporting</b></p> <p>Yes (low risk of bias) <input checked="" type="checkbox"/></p> <p>No (high risk of bias) <input type="checkbox"/></p> <p>Unclear (uncertain risk of bias) <input type="checkbox"/></p> <p><b>Other Potential Threats to Validity</b></p> <p>Yes (low risk of bias) <input checked="" type="checkbox"/></p> <p>No (high risk of bias) <input type="checkbox"/></p> <p>Unclear (uncertain risk of bias) <input type="checkbox"/></p>	<p>By pharmacy</p> <p>By pharmacy</p> <p></p> <p></p> <p></p> <p></p> <p></p>
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Study: **Young 1996 (S146)**; Single Centre Trial (SCT) / Randomized, Prospective, Double-blind, Controlled trial

Criteria used to assess methodological quality <sup>152</sup>				Cochrane "Risk of bias" assessment tool <sup>11</sup>	
Criteria	Score			Comments (additional)	Comments (additional)
	0	1	2		
Randomization	...	Not concealed or not sure	Concealed randomization	Not adequately described; not sure	Randomization table used
Blinding	Not blinded	...	Adjudicators blinded	Double-blind	
Analysis	Other	...	Intention to treat		
Patient selection	Selected patients or unable to tell	Consecutive eligible patients	...	Unable to tell	
Comparability of groups at baseline	No or not sure	Yes	...		
Extent of follow-up	<100%	100%	...		
Treatment protocol	Poorly described	Reproducibly described	...		
Co-interventions	Not described	Described but not equal or not sure	Well described and all equal		
Outcomes	Not described	Partially described	Objectively defined		
<b>Score: 12/14</b>					

Cochrane "Risk of bias" assessment tool <sup>11</sup>		Comments (additional)
<b>Sequence Generation</b>		
Yes (low risk of bias)	<input checked="" type="checkbox"/>	
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input type="checkbox"/>	
<b>Allocation Concealment</b>		
Yes (low risk of bias)	<input type="checkbox"/>	
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input checked="" type="checkbox"/>	
<b>Blinding</b>		
Yes (low risk of bias)	<input checked="" type="checkbox"/>	
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input type="checkbox"/>	
<b>Incomplete Outcome Data</b>		
Yes (low risk of bias)	<input checked="" type="checkbox"/>	
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input type="checkbox"/>	
<b>Selective Outcome Reporting</b>		
Yes (low risk of bias)	<input checked="" type="checkbox"/>	
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input type="checkbox"/>	
<b>Other Potential Threats to Validity</b>		
Yes (low risk of bias)	<input checked="" type="checkbox"/>	
No (high risk of bias)	<input type="checkbox"/>	
Unclear (uncertain risk of bias)	<input type="checkbox"/>	

**Appendix 6.8: Potentially eligible studies reported in languages other than English**

STUDY ID	STUDY	COMMENT
S70	Gärtner R, Angstwurm M. Die Bedeutung von selen in der intensivmedizin [Significance of selenium in intensive care medicine. Clinical studies of patients with SIRS/sepsis syndrome]. Med Klin 1999; 94(Suppl 3): 54-57.	Lack of randomization (not RCT?)
S82	Kuklinski B, Buchner M, Müller T, Schweder R. [Anti-oxidative therapy of pancreatitis-an 18-month interim evaluation]. Z Gesamte Inn Med 1992; 47: 239-245.	Lack of randomization (not RCT?)
S83	Kuklinski B, Buchner M, Schweder R, Nagel R. Akute Pancreatitis—eine free radical disease. Letalitätssenkung durch Natriumselenit (Na <sub>2</sub> SeO <sub>3</sub> )-Therapie [Acute pancreatitis--a "Free radical disease". Decreasing mortality by sodium selenite (Na <sub>2</sub> SeO <sub>3</sub> ) therapy]. Z Gesamte Inn Med 1991; 46: S145-S149.	Potentially eligible study
S84	Kuklinski B, Zimmermann T, Schweder R. Letalitätssenkung der akuten Pankreatitis mit Natriumselenit [Decreasing mortality in acute pancreatitis with sodium selenite. Clinical results of 4 years antioxidant therapy]. Med Klin 1995; 90(Suppl 1): 36-41.	Lack of randomization (not RCT?)
S86	Lehmann C, Egerer K, Weber M, Krausch D, Wauer H, Newie T, et al. Einfluss einer Selensubstitution auf verschiedene Laborparameter bei sepsisgefährdeten Patienten [Effect of selenium administration on various laboratory parameters of patients at risk for sepsis syndrome]. Med Klin 1997; 92(Suppl 3): 14-16.	Only provide outcomes for secondary objectives
S151	Zimmermann T, Albrecht S, Kühne H, Vogelsang U, Grützmann R, Kopprasch S. Selensubstitution bei Sepsispatienten. Eine prospektiv randomisierte Studie [Selenium administration in patients with sepsis syndrome. A prospective randomized study]. Med Klin 1997; 92(Suppl 3): 3-4.	Potentially eligible study