

The Effect of Perioperative Nutrient Status on Post-Radical Cystoprostatectomy
Complications

BY

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Abstract

Introduction

Radical Cystoprostatectomy (RC) is a major surgery for the treatment of muscle-invasive urinary bladder cancer with high postoperative complication rates. Evidence suggests surgeries such as RC induce arginine deficiency, leading to immunosuppression and morbidity. The supplementation of specialized immunonutrition (SIM), containing L-arginine, omega-3 fatty acids, RNA nucleotides, and vitamin A, has shown to improve postoperative outcomes in other surgical cohorts. The purpose of this project is to investigate the effects of perioperative SIM to meet nutrient demands and improve outcomes following RC.

Methods

Men undergoing RC at the University of Kansas Hospital (KUH) were randomized to receive either SIM (Impact Advanced Recovery®) or the oral nutrition supplement (ONS, Boost Plus®) drink. The study participants were blind to the intervention and consumed the supplements 5 days before and after surgery. Plasma arginine, citrulline, and ornithine concentrations were measured at days -6, 0, 2, 14, and 30. Early (0-30 days) and late (31-90 days) complications were graded based on the Clavien-Dindo scheme. Fisher's exact tests, T-tests, and linear mixed effect models were used to detect differences in outcomes.

Results

The SIM and ONS groups experienced 71% and 73% early complication rates ($P = 1$), and 14% and 43% late complication rates ($P = 0.21$), respectively. The SIM group had a non-significantly lower rate of antibiotic use in the late period (14%) compared to the ONS

group (50%, $P = 0.10$). Plasma arginine significantly decreased from baseline to POD2 ($P = 0.0003$) in the ONS group, with no significant change in the SIM group. There was no significant difference in plasma arginine and citrulline concentrations between the two groups over time ($P > 0.05$). There was a significant difference between in plasma ornithine concentrations between the two groups over time ($P = 0.04$). At the time of surgery, the SIM group had significantly higher plasma ornithine compared to ONS ($P = 0.001$).

Conclusion

The perioperative supplementation of SIM in RC patients induces a shift in metabolism of amino acids related to immune function and wound healing. Though SIM supplementation was unable to fully replete arginine status by surgery, the supplementation prevented a further depletion in plasma arginine following surgery. The trend toward lower infection rates in the SIM group suggests preserved immune function. A greater amount of arginine may be necessary to fully replete this population and optimize immune function following surgery. Additionally, the SIM supplementation produced an increase in plasma ornithine concentration at the time of surgery, which may enhance wound healing. More research is needed to confirm the benefits of SIM in the RC cohort.

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Chapter 1-Introduction

Urothelial bladder cancer is the fourth leading cause of cancer in men (1). Radical cystoprostatectomy (RC), the surgical removal of the urinary bladder, pelvic lymph nodes, distal ureters, urethra, intrapelvic nerves, prostate, and seminal vesicles, is the standard of care treatment for muscle-invasive urinary bladder cancer in men (2, 3). Unfortunately, surgery often leads to muscle wasting, weight loss, and postoperative complications (4, 5). Overall, complication rates of up to 64% have been reported within the first 90 days of surgery, with gastrointestinal and infectious complications being the most common (4).

While many factors may contribute to complications following RC, the use of Specialized Immunonutrition (SIM) in similar cohorts suggests that supplementation to meet the unique nutritional demands of surgery may improve postoperative outcomes. Studies of gastrointestinal surgeries found a decrease in total complications, infections, and hospital length of stay with the use of SIM (6-10). While little investigation has been done in the RC cohort, in a case-control pilot study of RC patients, Bertrand et al. (11) found a 36.7% decrease in complication rates with preoperative SIM, including a significant decrease in ileus and infections.

The immunonutrients in SIM include L-arginine, omega-3 fatty acids, vitamin A, and RNA nucleotides. While each of these immunonutrients likely has pleiotropic and synergistic effects, evidence suggests L-arginine may play the most significant role. Arginine is a conditionally essential amino acid that is depleted following physical traumas (12-14). Though the exact mechanism is unknown, this may occur from an over-expression of arginase 1 by immature, immunosuppressive cells called myeloid-derived suppressor cells (MDSC) (12, 14). The arginine deficiency and increase in MDSC and arginase 1 are

believed to contribute to poor adaptive immunity through impaired T lymphocyte function, including reduced cellular memory, proliferation, and cytotoxicity (12, 14). Low plasma arginine may also increase susceptibility to complications due to its role in wound healing, inflammation regulation, and bactericidal action, and has been termed “arginine deficiency syndrome” (15-17). The decrease in intracellular arginine and impaired T-cell function with coinciding poor adaptive immunity has been seen in vivo following physical injury (14). Murine models that suffered from the physical injury experienced greater bacterial growth and mortality from an induced infection when compared to the non-injured model. A similar response to infection was seen in non-injured models injected with MDSC or arginase (14). However, immune function was restored in injured subjects with administration of an arginase inhibitor, further implicating arginine’s role in the mechanism (14).

Research suggests supplementation can restore arginine status. SIM supplementation in the critically ill has shown to significantly increase plasma arginine after 7 days, while ornithine, an amino acid metabolite of arginine, significantly increased after 24-hours of supplementation. This indicates arginine supplementation may impact metabolism even prior to a reflection in plasma concentration (18). *In vitro*, arginine improves T lymphocyte function previously impaired by MDSC exposure (12, 19). While Ochoa et al. (20) found low nitric oxide metabolite levels in trauma patients, supplementation of L-arginine has also shown to increase nitric oxide production and muscle viability in ischemic tissue in porcine models (21). Arginine supplementation, therefore, may prevent postsurgical complications by restoring immune function and improving wound healing.

Many studies continue to show the benefits of SIM and the role of arginine in trauma recovery; however, the efficacy of SIM to meet arginine demands and prevent complications following RC has yet to be established.

STATEMENT OF PURPOSE: To investigate the effect of perioperative SIM supplementation on arginine status and postoperative complications in the RC cohort.

RESEARCH QUESTIONS:

1. Does perioperative SIM supplementation reduce the quantity and/or severity of postoperative complications following radical cystoprostatectomy?
2. Does perioperative SIM supplementation prevent arginine deficiency (<80 umol/L) at either surgery or postoperative day 2?
 - a. Does plasma arginine at the time of surgery correlate with postoperative complications?
 - b. Does plasma arginine at postoperative day 2 correlate with postoperative complications?
3. Does perioperative SIM supplementation change plasma arginine, ornithine, or citrulline levels at the time of surgery or postoperative day 2?

Chapter 2-Review of Literature

INTRODUCTION

Radical cystoprostatectomy, considered the gold standard for muscle-invasive bladder cancer (2), is a complicated procedure with considerable morbidity rate, seen as high as 64% in the first 90 days (4). While several factors contribute to postoperative complications, optimizing nutritional status proximate to RC may be a key component to improving outcomes.

The nutritional needs in the RC cohort are multifaceted. Prior to urologic surgeries, 16-47% of patients are at risk for malnutrition, which has shown to impact complication rates (22-24). The catabolic impact of surgical trauma increases the nutrient demand, leaving patients nutritionally deplete. Theories are arising suggesting induced micronutrient deficiencies impact immune function and contribute to the complications following surgery (12).

To meet these unique nutritional needs, supplementation of L-arginine, omega-3 fatty acids, vitamin A, and nucleotides through specialized immunonutrition (SIM) has been used to optimize surgical recovery (25). Each component of SIM has a particular role; however, L-arginine, with its effect on modulating immune function, may be the most pertinent in improving postoperative outcomes.

Currently, there is a gap in the literature involving the use of SIM in RC patients. The nutritional needs of RC patients, efficacy of SIM in similar cohorts, and preliminary data supporting the role of nutrition in RC outcomes give evidence for the potential use of SIM in this cohort (11, 26-28). However, currently the data for SIM use in urology are lacking (26).

More research is needed to determine the effect of perioperative SIM on improving outcomes in RC.

BACKGROUND

Urinary bladder cancer is among the most common malignancies in the United States. Urinary bladder cancer is the fourth leading cause of cancer incidence and eighth leading cause of cancer-related death in men (1). The National Cancer Institute's Surveillance, Epidemiology, and End Points Report (SEER) estimates 74,000 new cases and 16,000 deaths related to urinary bladder cancer in 2015 (1).

Many factors appear to increase the risk of bladder cancer. Men are four times more likely to get bladder cancer than women (29). Genetic factors, particularly those involved in detoxification pathways such as genotypic variants in N-acetyltransferase and glutathione-s-transferase mu 1 genes increase risk for bladder cancer (30). Smoking causes about half of all urothelial tumors with a fourfold increase in risk for current smokers (31). Occupational exposures such as aromatic amines, polycyclic aromatic hydrocarbons, and chlorinated hydrocarbons may contribute to about 20% of urothelial bladder cancers (30). Fluid intake and subsequent carcinogen exposure in tap water may also play a role, but remains controversial due to the perceived benefits of urine dilution and frequent urination (30). Exposures to environmental heavy metals, such as arsenic and cadmium, have also shown to increase bladder cancer risk (32, 33).

Bladder cancer is detected at various stages and severities, generating varying effects on treatment plan and prognosis. Bladder cancer is staged based on cell type, depth of tissue layer penetration, lymph node involvement, and distant metastasis. For superficial

bladder cancer confined to the mucosa or lamina propria, the typical regimen includes tumor resection with continued surveillance (2). However, due to the risk of progression, many patients undergo intravesical therapy or proceed to the RC for high-risk superficial tumors (2). Among all stages, there is a 77.9% survival rate at five years following diagnosis (29). This survival rate drops to 70.2% for invasive tumors (29). Fifty percent of bladder cancer cases will ultimately involve distant metastasis following surgery. For this reason neoadjuvant or adjuvant chemotherapy is often employed increasing survival rate 5.0-6.5% (2).

For those with muscle-invasive bladder cancer, RC is the standard treatment (2). While technical variations exist, RC typically involves extraction of the urinary bladder, pelvic lymph nodes, distal ureters, urethra, intrapelvic nerves, prostate, and seminal vesicles in men (3). Urinary diversions can be either incontinent, commonly the ileal conduit, or continent, commonly orthotopic neobladder, both requiring reconstruction of the bowel (3, 29, 34, 35). Following surgery, patients often suffer from urinary incontinence, sexual dysfunction, and other side effects impacting quality of life (35, 36). While the orthotopic neobladder is typically the favored diversion, certain contraindications such as positive urethral margins may cause preference to the quicker and easier ileal conduit (35). Recently, a study comparing the two diversions found that the neobladder led to improved quality of life including physical and social functioning (37). Among all techniques, the mortality rate following RC is still relatively high, shown to be 2 and 7% at 30 and 90 days, respectively, in a large contemporary series (38). The burden of bladder cancer and subsequent surgery gives reason for investigating ways to improve outcomes in this cohort.

COMPLICATIONS TO THE RADICAL CYSTOPROSTATECTOMY

While many different complications can occur, gastrointestinal and infectious complications are the most common and often the most severe following RC (4). Shabsigh et al. (4) found in a large retrospective review that gastrointestinal complications occur in 29% of patients, most commonly from ileus. A smaller study confirmed ileus to be the most common minor complication, contributing to prolonged hospital stay following RC and correlating with a higher occurrence of major complications (39, 40). The cohort examined by Shabsigh et al. also found a quarter of patients experience infectious complications, including urinary tract infections, sepsis, and wound infections (4). When considering only the more severe complications, gastrointestinal and infectious complications continued to be the top contributors in this large cohort (4). These two complications are also implicated in cause for readmissions. Readmission rates in the first 90 days following RC have been seen at 26%, most commonly due to ileus, pyelonephritis, and urinary tract infection (38).

The inflammatory response following RC is a likely culprit in these postoperative complications, an underlying mechanism in both ileus and infection (41, 42). The systemic inflammatory response syndrome (SIRS) is non-distinct inflammation diagnosed clinically by the presence of two or more of the following: (1) fever or hypothermia, (2) tachycardia, (3) tachypnea, (4) leukocytosis or leucopenia (43). Along with the clinical signs, SIRS is often accompanied by a change in inflammatory cytokines such as tumor necrosis factor (TNF)- α , interleukin (IL)- 1, -6, and -10 and immune function (44). SIRS occurs at a higher frequency in major operations and presence beyond 72-hours postoperatively has been predictive of complications following gastrointestinal surgery (43, 45). A significant rise in

IL-6 has been seen following RC, along with a frequency of SIRS over 70% (44). Mitigating this inflammatory response following RC may improve immune function and minimize postoperative complications.

Grading Complications

Historically, there have been inconsistencies in reporting RC complications (46). The development of the Clavien-Dindo grading system in 2004 gave the surgical field a mode of standardized reporting (47). The scheme grades any deviation from the ideal postoperative course, based on the treatment severity, with a grading scale from I (least severe) through V (death). This grading scheme minimizes the subjectivity of determining minor versus major complications in reporting surgical outcomes. Clavien et al. (48) conducted a study five years following the proposed grading system evaluating the use and further clarifying points of inconsistency. The authors made one clarification to move away from ambiguous terms such as “minor” and “major” and use the grading scheme as originally indicated without variation (48). The lack of standardized reporting could be reason for variability in morbidity and must be considered when reviewing the literature for complications in RC. Using the standardized Clavien-Dindo system will bring validity and reliability to reporting complications in the RC cohort.

MALNUTRITION AND RADICAL CYSTOPROSTATECTOMY

Following major surgery, such as RC, proper wound healing and preservation of muscle mass requires adequate calories and protein (13). Mathur et al. (5) reported an increased caloric expenditure of 11% above expected, in a small trial in men two weeks following RC. The investigators also found a 7% loss in total body protein and reduced

muscle strength at the same time point (5). Six months later, the muscle strength returned completely; however, only 63% of the lost body protein was regained (5). Hensle et al. (27) demonstrated a nitrogen loss occurs following RC regardless of nutritional support (5% dextrose versus total parenteral nutrition containing amino acids). However, the nitrogen balance was restored only in the group receiving amino acids by postoperative day 4. The group receiving 5% dextrose lost significantly more nitrogen per kilogram of body weight in the 6 days monitored in the trial, indicating the importance and efficacy of nutritional support in body protein preservation (27).

Due to the catabolic response of the surgery, nutritional status prior to RC may affect outcomes. In general, malnutrition is associated with poor surgical outcomes including an increased length of stay, elevated critical care admission rates, prolonged wound healing, and infection (13). In hospitalized urologic cohorts, 16-21% of patients are at severe risk of being malnourished (22, 24), with higher rates, 33-46%, seen in those undergoing RC and open surgeries (22, 23). The reported studies utilized the Nutritional Risk Screening tool that assesses weight loss, body mass index (BMI), and food intake (22-24). A score indicating high risk of malnutrition is correlated with a three times greater likelihood for complications following a urologic surgery (23). Another study specifically conducted in RC patients, using albumin, BMI, and pre-surgical weight loss, found 19% of patients are malnourished, which increased risk for early (90 days postoperatively) mortality and predicted a poor 3-year survival rate (28). These studies indicate the need for nutritional support surrounding RC for an optimal postoperative course.

Measuring Malnutrition

Many different assessments are used in determining nutritional status. Body mass index, recent weight loss, change in food intake, and preoperative albumin are commonly used in RC cohorts. The use of albumin as a nutritional marker is controversial, and may indicate disease severity rather than protein status (49). The American Society of Parenteral and Enteral Nutrition (ASPEN) recommends a standardized system for assessing nutritional status in oncology patients using weight loss, estimated energy consumption, and physical findings (50). Several assessments for malnutrition including evaluations of physical findings have been validated in oncology patients. One of these is the patient generated-subjective global assessment (PG-SGA). The PG-SGA includes assessing depletion of muscle and fat stores as well as fluid accumulation (51). The findings from the PG-SGA can be used to assess malnutrition in the RC patients, employing the ASPEN criteria to draw conclusions between nutritional status and postoperative outcomes.

SPECIALIZED IMMUNONUTRITION

The nutritional demand of RC extends beyond the need for calories and protein. The evidence of catabolism following RC surgery presents a case for early nutrition support in preventing loss of lean mass; however, administration of conditionally essential nutrients through specialized immunonutrition (SIM) may confer added benefit in improving outcomes. While many variations of immunonutrition exist, one particular supplement, Impact Advanced Recovery®, has shown high efficacy in many different cohorts. The nutrients of interest in Impact Advanced Recovery® include L-arginine, omega-3 fatty

acids, vitamin A, and nucleotides. Animal and human studies have given rise to proposed mechanisms of action for these nutrients, and clinical trials have suggested their clinical significance that may lead to improved surgical outcomes in RC patients.

Arginine

Arginine is an amino acid made endogenously and in sufficient supply under normal conditions. The average daily intake is 4-6g; however, the human body makes about 15-20g per day through the citrulline intestinal-renal axis to maintain plasma concentrations of 80-100 $\mu\text{mol/L}$ (15, 52). Under stressful conditions such as illness or following a major surgery, plasma levels fall, therefore requiring exogenous production and giving arginine the classification as a conditionally essential amino acid (13). In fact, arginine depletion by 50% or greater to a level of deficiency following surgery or trauma has been reported in the literature (12).

Arginine is mostly used in protein synthesis for cell growth and proliferation; however, arginine is also metabolized by nitric oxide synthase (NOS), to produce nitric oxide (NO) and citrulline, and arginase, to produce ornithine and urea (Figure 1) (17, 52). The role of NO is differentiated by the synthase active in its production. Nitric oxide can act as a neurotransmitter, vasodilator, or immune regulator to kill infectious pathogens, modulate inflammation, and aid in T lymphocyte proliferation (15). Arginine metabolism by arginase may promote wound healing through the ultimate production of proline and polyamines from ornithine (17). Both of the major enzymes for arginine have inducible forms that are active in response to different T helper cells and their cytokines, differentiating arginine's role based on the immune state of the body (17). The role of arginine in T lymphocyte function is continuing to be studied. T lymphocytes require

adequate arginine for cellular memory, production of membrane receptors, and proper cell cycle (17, 53, 54). Due to its role in wound healing and immune function, inadequate arginine may leave a patient at greater susceptibility for complications.

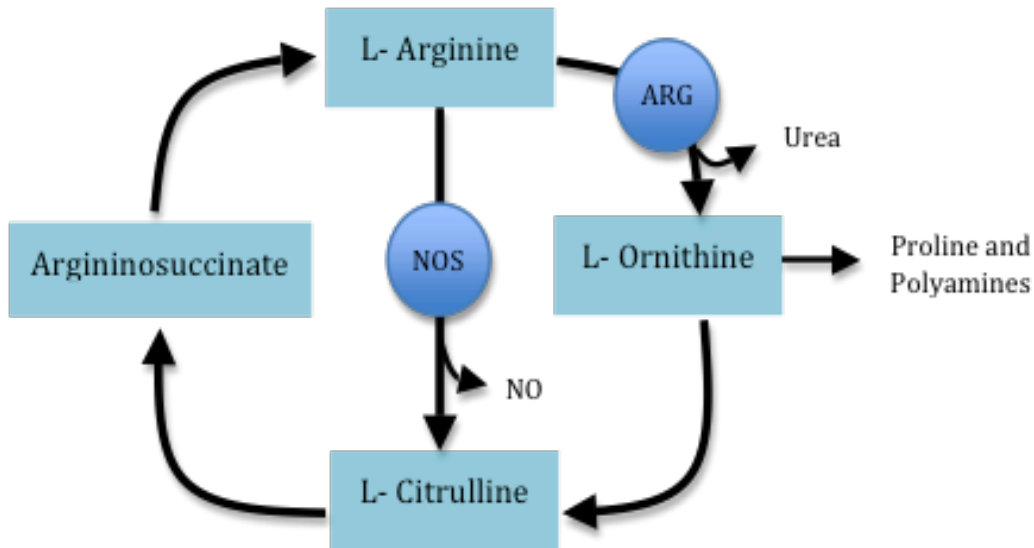


Figure 1- Arginine metabolism. NOS= nitric oxide synthase; NO= nitric oxide; ARG= arginase

Zhu et al. (12) compiled a proposed mechanism of action of arginine as an immunonutrient based on animal trials within their laboratory. Based on their findings, the depletion of arginine following cellular stress appears to be caused by its increased breakdown, and can be observed within hours of surgery or trauma. Myeloid cells increase following tremendous physical stress. These myeloid cells are unique in that they are immature and over-express arginase 1. Under normal conditions, myeloid cells typically do not metabolize much arginine; however, cellular stress appears to activate high-affinity cationic amino acid transporters that shuttle arginine into myeloid cells, depleting its availability to T lymphocytes (17). These myeloid cells inhibit T-cell activity in vitro, and may be cause for the immunosuppression following trauma (19). Because of their immunosuppressive activity, these cells that over-express arginase 1 have been termed

myeloid-derived suppressor cells (MDSC) (12). However, the inhibition on T lymphocyte function is reversed with either an arginase antagonist or L-arginine supplementation, suggesting nutritional intervention may restore immune function (19).

Arginine may also have specific implications in sepsis, which would be of interest for the RC cohort. During sepsis, there is a decrease in plasma arginine (13). Based on the proposed mechanism, low arginine due to upregulated MDSCs may leave an individual at greater susceptibility for infection due to the T lymphocyte suppression. However, inconsistency in human trials have lead to controversy over arginine supplementation in sepsis (52).

Nutritional status prior to injury may influence arginine metabolism, as well. A study in a porcine model showed that reduced caloric intake prior to sepsis led to a greater decrease in arginine levels, nitric oxide, and protein synthesis compared to the adequately nourished group (55). These findings suggest caloric restriction may worsen arginine depletion and nitric oxide production, potentially further impairing recovery after trauma. Conversely, another study in a porcine model demonstrated the supplementation of L-arginine can lead to increased nitric oxide and muscle viability to an otherwise ischemic tissue (21). Therefore, optimizing nutritional status prior to trauma or illness may improve outcomes.

The depletion of plasma arginine, increase in arginase 1, decrease in NO production, T lymphocyte dysfunction, and biological consequences have been collectively termed arginase deficiency syndrome (ADS) (17, 54). Cancer, surgery, and trauma patients exhibit signs of ADS, therefore becoming subjects of study for arginine supplementation (17).

Drover et al (9) conducted a meta-analysis of randomized control trials in the perioperative

supplementation of arginine in surgical patients. Trials involving arginine with or without other nutrients were included. Overall, there was a significant decrease in infectious complications and hospital length of stay compared to controls, with even greater efficacy for the Impact formulation and perioperative supplementation (9).

The state of arginine deficiency following a surgical trauma appears to put RC patients in a vulnerable state for morbidity from infection and ischemia. The exogenous intake of L-arginine to meet the increased demand may restore immune and wound healing function in the RC cohort to prevent postoperative complications.

Omega-3 Fatty Acids

Omega-3 fatty acids (n-3) are one of the two classes of fatty acids required from exogenous sources. There are three different n-3s of metabolic importance including alpha-linolenic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Alpha-linolenic acid is the only truly essential n-3 and can be converted to the EPA and DHA in the body by the delta-6 and 5 desaturase enzymes. However, desaturase enzymes are variable in conversion efficiency, making EPA and DHA important dietary constituents as well (56). The major dietary sources of EPA and DHA are fatty fish such as mackerel or salmon (57). Fish oils are used in SIM to add the immune-modulating n-3.

The immune-modulating effects of n-3 are likely due to the anti-inflammatory effects. The other essential class of fatty acids are omega-6 fatty acids (n-6). Omega-6 fatty acids have mostly pro-inflammatory effects. Fatty acids of both families are embedded into cellular membranes in limited storage. During times of cellular stress, phospholipase A₂ cleaves available membrane fatty acids, which are then further metabolized by the induced cyclooxygenase and lipoxygenase enzymes. Arachidonic acid (AA), an n-6 fatty acid, is

metabolized to form 2 and 4 series eicosanoids including prostaglandins E₂ (PGE₂), which have pro-inflammatory action. Conversely, EPA is metabolized to the 3 and 5 series eicosanoids, including prostaglandin E₃ (PGE₃). The 3 and 5 series eicosanoids are less inflammatory and actually counteract the 2 and 4 series eicosanoids. Therefore, since the two types of fatty acids compete for space in the cellular membrane and metabolism by common enzymes to produce eicosanoids of counter-effects, their balance is crucial in determining the level of systemic inflammation (16). Additionally, the EPA and DHA derivatives, protectins and resolvins, may reduce the inflammatory phase and prevent the associated damage (58).

Omega-3 fatty acids may have action in conjunction with arginine regarding MDSCs. Following injury, evidence suggests omega-3 fatty acids may restrain the rise in MDSCs (12). While all prostaglandin series induce arginase 1, PGE₃ does so markedly less than the n-6 prostaglandin series, indicating a greater depletion of arginine from the metabolism of n-6 compared to n-3 in response to cellular stress (59).

Optimally, n-3 and n-6 would be present in cellular membranes in a proper ratio to allow for an appropriate level of inflammation following a stress response. However, the typical consumption of n-3 in the Western diet of less than 0.2g/day is about 15-20 times less than the typical n-6 consumption (57). Therefore, it is likely the RC patients are entering surgery with membrane fatty acid composition favoring hyperinflammation. By supplementing omega-3 fatty acids perioperatively in RC patients, the inflammation may be reduced resulting in fewer complications.

Efficacy in supplementing n-3 through an oral nutrition supplement around surgery is still being investigated. Sorensen et al. (60) conducted a double-blinded placebo-

controlled trial looking at perioperative n-3 supplementation for colorectal cancer surgery. The study found no changes in complication rates or hospital length of stay with the intervention. However, in a meta-analysis of SIM in the critically ill, a subgroup analysis indicated that the fish oil containing supplements had added benefit of decreased mortality and reduced length of stay (61). Aida et al. (62) found SIM supplementation before a major surgery resulted in higher EPA/AA ratios, reduced PGE₂ production, and ultimately a lower rate of infectious complications. While more investigation is necessary for the role of omega-3 fatty acids in improving post-surgical outcomes, this evidence suggests supplementation with other immune-modulating ingredients such as L-arginine is most efficacious.

Nucleotides

Under normal conditions, the body produces nucleotides in de novo production or recycles them from cell turnover. That being said, about 1-2 g of nucleotides are additionally absorbed each day through dietary sources such as fruits and vegetables, organ meats, seafood, and dried legumes (63).

Nucleotides have many functions crucial for growth and metabolism. Nucleotides are the building blocks for DNA and RNA necessary for storing and transferring genetic information, cellular division, and protein synthesis. Units of cellular energy, adenosine triphosphate and uridine triphosphate, are both made of nucleotides. Additionally, components of the secondary messenger system used for cell signaling and enzyme regulation, cyclic adenosine monophosphate and cyclic guanosine monophosphate, rely on nucleotides (63).

During times of increased protein demand such as gastrointestinal insult, rapid growth, and immunocompromised situations, nucleotide depletion may occur (63). For this reason, it has been said that nucleotides become semi-essential in a diseased state (64). Due to the functional role of nucleotides in basic cellular processes, inadequate availability could have adverse consequences, particularly in cells of rapid turnover like the alimentary tract and immune system (63). Considering the high proportion of GI and infectious complications in the RC cohort (4), maintaining nucleotides may impact postoperative outcomes.

While human studies of nucleotides typically include the use of other components in SIM, many animal models have looked at nucleotides specifically. In one study, Xu et al. (64) found nucleotide supplementation enhanced innate and acquired immunity in healthy mice.

Due to the catabolic nature of RC, an increased need for nucleotides following surgery is likely. While the evidence of nucleotides alone in affecting surgical outcomes is limited, proposed mechanistic action and its presence in successful SIM interventions suggest exogenous intake of nucleotides may improve outcomes in the RC population.

Vitamin A

Vitamin A is a fat-soluble vitamin required through the diet. Animal sources such as liver, eggs, and fish carry the preformed retinyl palmitate. Vegetables, particularly those of dark green and deep orange color, contain carotenoids that can be used by the body to synthesize retinoic acid, a vitamin A metabolite (65). Retinoic acid and beta-carotene are found in SIM.

Vitamin A has many roles pertinent to surgical recovery. Vitamin A plays a role in tissue repair via gene regulation during the cell cycle, as demonstrated in the effects of retinoic acid deficiency, where the epithelial lining becomes keratinized. Vitamin A also has a role in immunity by promoting T-helper 2 cell formation. For this reason, immune function decreases during deficiency (15). Retinoic acid has another role when considering again the presence of MDSCs in the stress response. Retinoic acid appears to mature MDSCs which arrests their inhibition on T-cells (12, 66).

Retinoic acid may be of particular interest for the RC cohort by reducing urinary tract infections. The addition of either vitamin E or A to standard antibiotic treatment brought reduced renal scarring in children with acute pyelonephritis (67). Patients with recurrent lower urinary tract infections saw a reduction in infections after adding vitamin A to their standard antimicrobial (68).

The evidence supporting vitamin A's role in immunity, particularly with infections of the urinary tract, illustrates its potential efficacy in reducing postoperative complications in RC patients.

Specialized Immunonutrition in Surgery and Infection

While each component of SIM offers unique value in abating inflammation and improving immunity, the combination of them into one formula has the best evidence for influencing outcomes.

Numerous trials have shown the efficacy of SIM in reducing inflammation after surgery. In a study done in a cardiac surgery cohort, patients receiving the SIM showed a reduced IL-10 expression (69). A blunt in the rise of Il-6 from L-arginine and omega-3 supplementation after colorectal surgery has also been seen (70). Both IL-6 and 10 are T

helper 2 cytokines, which have been implicated as inducers of arginase 1 (17, 71).

Therefore, a change in these inflammatory markers from SIM may lead to a lower induction of arginase 1 and a subsequent favorable immune response.

As a result of the modulation of inflammatory response, SIM supplementation has shown to improve postoperative outcomes. Reduction in hospital length of stay, infectious complications, and overall complication rates have been seen in many oncologic surgical populations, including pancreatic, colorectal, and stomach cohorts (72, 73). However, due to the gastrointestinal (GI) reconstruction during RC, the studies using SIM in GI cohorts may be the most relevant. Osland et al. (6) conducted a meta-analysis of immune-modulating nutrition in major elective GI cancer surgeries. Twenty-two placebo-controlled trials were included with 11 using the SIM formulation and 6 solely using arginine. The study found a significant decrease in infectious complications and hospital length of stay for both perioperative and postoperative supplementation. A Cochrane review of GI surgeries found a 15% reduction in total complications and 13% reduction in infectious complications with preoperative immunonutrition compared to either a placebo or conventional management (8). Marimutha et al. (7) conducted a meta-analysis over immunonutrition in major open GI surgeries for only randomized control trials using an isocaloric/isonitrogenous control. The investigators reported a significant reduction in length of stay by almost two days and a 35% reduction in postoperative complications. Findings such as these prompted the European Society for Parenteral and Enteral Nutrition to recommend perioperative administration of immune-modulating formulas for neck and abdominal cancer patients (74).

While many of the studies involving SIM are similar, some heterogeneity exists. Evidence supports that both L-arginine and omega-3 fatty acids must be present within the supplement to exert the postoperative benefits (25). Furthermore, there is higher efficacy for the Impact formulation as compared to other immune-modulating formulas (25). Additionally, when stratified for the time of intervention, it appears perioperative supplementation confers a greater reduction in complication rate and length of stay (7). Therefore, perioperative supplementation with a complete formulation during course may be most effective at modulating inflammation and preventing complications in the RC cohort.

Limitations in the Literature

Successful findings continue to be replicated in many surgical or critical care cohorts; however, as pointed out in the review of literature by Munbauhal et al. (26), data are lacking for the use of SIM in urology cohorts. Bertrand et al. (11) conducted the first intervention study in a multi-centric pilot case-control study with preoperative SIM supplementation. The findings were promising with a postoperative complication rate of 40% in the SIM group compared to 76.7% in the control. Additionally, there was an increase in severity of complication in the control group, and a trend in a decreased length of stay of 3 days in the SIM group. Unfortunately, the trial lacked randomized placebo control, showed heterogeneity in the multi-centric approach, and used a retrospective review for the controls. Furthermore, the intervention was conducted strictly preoperatively, and nutritional status was not measured either before or after the intervention (11). For this reason, more research is needed in the use of perioperative SIM supplementation in the RC cohort.

CONCLUSION

The high complication rate following RC remains a concern to be addressed (4). These complications may be worsened by suboptimal nutritional status. Many RC patients approach surgery already nutritionally deplete. The catabolic surgery increases the need for many nutrients, leading to deficiencies that can aggravate postoperative complications. The mechanistic evidence of the individual nutrients and the efficacy of the combined formulation show great promise in the use of SIM to restore immune function and improve surgical outcomes. The investigation of SIM in reducing postoperative complications has been a success in many other cohorts; however, data are still lacking for the RC cohort (25, 26, 61). Due to the nutritional need of the patients and lack of evidence for the use of SIM, the RC cohort should be investigated for the effect of perioperative SIM on post-surgical outcomes. Our study will measure the ability of the perioperative SIM supplementation to meet the nutritional demands of RC in order to prevent postoperative complications.

Chapter 3-Methods

Study Design

The study was a prospective, randomized, single-blinded placebo-controlled pilot clinical trial. The study took place at the University of Kansas Medical Center between September 2013 and April 2015. The Human Subjects Committee of the University of Kansas Medical Center Institutional Review Board and the protocol review monitoring committee of the University of Kansas Cancer Center approved the study protocol. Individuals with locally advanced urothelial bladder cancer scheduled for radical cystoprostatectomy at the University of Kansas Hospital (KUH) were contacted and screened for eligibility. Exclusion criteria included swallowing difficulties, metastatic disease, weight loss $\geq 10\%$ six months prior to surgery, body mass index (BMI) ≤ 18.5 , active viral infections, history of gouty arthritis or uric acid stones, allergies to milk, soy, or fish, female gender, undergoing cystectomy for non-bladder malignancy, and presence non-urothelial cancer. Written informed consent was obtained for all participants prior to enrollment.

Participants completed study visits at baseline and postoperative days (POD) 14 and 30. Data for the patient-generated subjective global assessment (PG-SGA) (51) and Charlson comorbidity index (75) were gathered at baseline.

Supplementation

Participants on the intervention arm received specialized immunonutrition (SIM, Impact Advanced Recovery®) nutrition drinks, and those on placebo arm received the iso-caloric/iso-nitrogenous oral nutrition supplement (ONS, Boost Plus®) nutrition drink. The

nutrient composition of the nutrition drinks is described in Table 1. The study team wrapped all nutrition drinks in opaque tape to preserve the single blinding. The study team administered 15 drinks to each participant with instructions to consume three each day for five days prior to surgery. Within 24 hours of surgery after physician approval, the study team initiated feeding with the nutrition drinks. Postoperative feeding began with 60mL boluses of the drinks. The participants received a total of 15 drinks after surgery and were instructed to consume up to three drinks a day as tolerated until all drinks were finished. The study team visited the participants during their hospital stay to coach patients on oral consumption strategies including regular ambulation. Diet orders were placed by the physician team and advanced as indicated. Per study protocol, KUH dietitians monitored all participants.

Participants completed diet diaries detailing the time and amounts of the nutrition drink consumed, along with any side effects. The study team monitored compliance by collecting the diet diaries along with the nutrition drink cartons.

Table 1- Composition of nutritional supplements¹

	Specialized Immunonutrition	Oral Nutrition Supplement
Name	Impact Advanced Recovery®	Boost Plus®
Energy (kcal)	1020	1080
Protein (g)	54.0	42.0
Arginine	14.1	1.8
Nitrogen	8.6	6.7
Fat (g)	27.6	42.0
Omega-3 fatty acids	3.3	0.0
Carbohydrates (g)	135.0	135.0
Fiber (g)	10.8	9.0
Vitamin A (IU) ²	5670	3750
RNA (g)	1.2	0.0

¹Intake reflective of one day of supplementation (711mL)

²Includes 50% of vitamin A activity from beta-carotene

Amino Acids

Whole blood was collected in sodium EDTA tubes at baseline (greater than 5 days prior to surgery), 3 hours after surgical incision, and on POD 2, 14, and 30. Plasma was collected and stored at -80°C after centrifugation of the whole blood samples (180 x g for 5 min at 25°C).

The amino acid analysis was performed using the Ultrapformance® liquid chromatography with ultraviolet detection at the Institute for Metabolomic Disease, Baylor Research Institute. The samples were analyzed in two batches, with all samples from a single participant contained within the same batch. An internal control was used for determining batch variability. Interassay variability was between 1.6 and 2.3% for arginine, 2.5 and 2.7% for ornithine. Sufficient levels of plasma arginine were defined as $\geq 80 \mu\text{mol/L}$ based on the reference range 80-100 $\mu\text{mol/L}$ (15).

Postoperative Complications

Following surgery, participants were monitored using the KUH electronic medical record for postoperative complications. When appropriate, outside medical records were requisitioned to assess complications treated outside of KUH. Early (within 30 days postoperatively) and late (31-90 days postoperatively) complications were recorded and graded using Clavien-Dindo scheme as described in Table 2 (47). All complications occurring within the time frame were graded regardless of a perceived likelihood of relationship to the radical cystoprostatectomy, as recommended by authors of the classification scheme (48). In addition to grading, complications were defined using surgery-specific categories (ileus, deep vein thrombosis, pneumonia, wound infection, urinary tract infection, return to operation room, pulmonary embolus, myocardial infarction, cerebral vascular accident, dehiscence, sepsis, respiratory failure, bowel leak, urine leak, small bowel obstruction, death, or other). Postoperative ileus was defined as a delay in feeding of greater than or equal to five days postoperatively. Infectious complications were identified by the prescription of non-prophylactic antibiotics. Readmission rates were recorded along with the incidence of systemic inflammatory response syndrome (SIRS) as defined in Appendix A (76). Complications were graded and recorded under the advisement of Dr. Eugene Lee, Assistant Professor in the Department of Urology Surgery. Age, gender, smoking history, comorbidities, and pathological information pertaining to participants' perioperative and postoperative courses were recorded in REDCap.

Table 2 - Clavien-Dindo grading scheme for the classification of surgical complications (47)

Grades	Definition
Grade I	Any deviation from the normal postoperative course without the need for pharmacological treatment or surgical, endoscopic and radiological interventions.
Grade II	Allowed therapeutic regimens are: drugs as antiemetic, antipyretics, analgesics diuretics, electrolytes and physiotherapy. Requiring pharmacological treatment with drugs other than such allowed for grade I complications. Blood transfusions and total parenteral nutrition are also included.
Grade III	Requiring surgical, endoscopic or radiological intervention
Grade IIIa	Intervention not under general anesthesia
Grade IIIb	Intervention under general anesthesia
Grade IV	Life-threatening complications (including central nervous system complications) requiring intermediate care or intensive care unit management.
Grade IVa	Single organ dysfunction (including dialysis)
Grade IVb	Multi organ dysfunction
Grade V	Death of a patient
Suffix "d"	If the patient suffers from a complication at the time of discharge, the suffix "d" (for "disability") is added to the respective grade of complication. This label indicated the need for a follow up to fully evaluate the complication.

Dietary Intake

Twenty four-hour dietary recalls were performed at baseline and postoperative days 14 and 30 (Table 5). The intakes were analyzed and verified using the Nutrition Data System for Research.

Statistical Analysis

Prior to outcome data analyses, baseline characteristics were summarized by means and standard deviations for numerical variables and by percentages and frequencies for categorical variables. Fisher's exact tests were used to detect the differences in the complication rates, complication grades, complication types, readmission rates, and arginine sufficiency between groups. T-tests were used to assess the differences between

continuous variables. Logistic regression was used to look at the association between requirement of antibiotic use and arginine levels at time of surgery and POD 2 as both a continuous and categorical (sufficient or deficient) variable. Linear mixed effect models with repeated measure were used to analyze the plasma amino acids over 5 time points. A significance level of $P < 0.05$ was set as statistically significant. Statistical analyses of the data were performed by Dr. Prabhakar Chalise, Assistant Professor in the Department of Biostatistics, using R and SAS 9.4 software.

Chapter 4- Results

Baseline Characteristics

Twenty-nine subjects were included in this study; 14 subjects were randomized to the SIM group and 15 were randomized to the ONS group (Figure 2). Patient characteristics are given (Table 1). Two participants from the ONS group voluntarily withdrew after completing the pre-operative supplementation for reasons unrelated to the supplement. However, any allowable data according to the signed consent form from the two participants were included in analysis. One participant in the ONS group is excluded from current analysis for late complications since he had yet to meet his POD 90 by the time of data extraction. Difficulties during blood draw and inability for several participants to attend follow-up study visits (POD 14 and 30) limited the available data at days 0, 2, 14, and 30; total number of participants (n) is given for each data set.

Participants in the SIM and ONS groups were similar in age (69.6 and 68.1 respectively). The two groups were similar in baseline characteristics with the exception of BMI (Table 3). Participants in the SIM group had a lower BMI compared to the ONS group ($P = 0.04$).

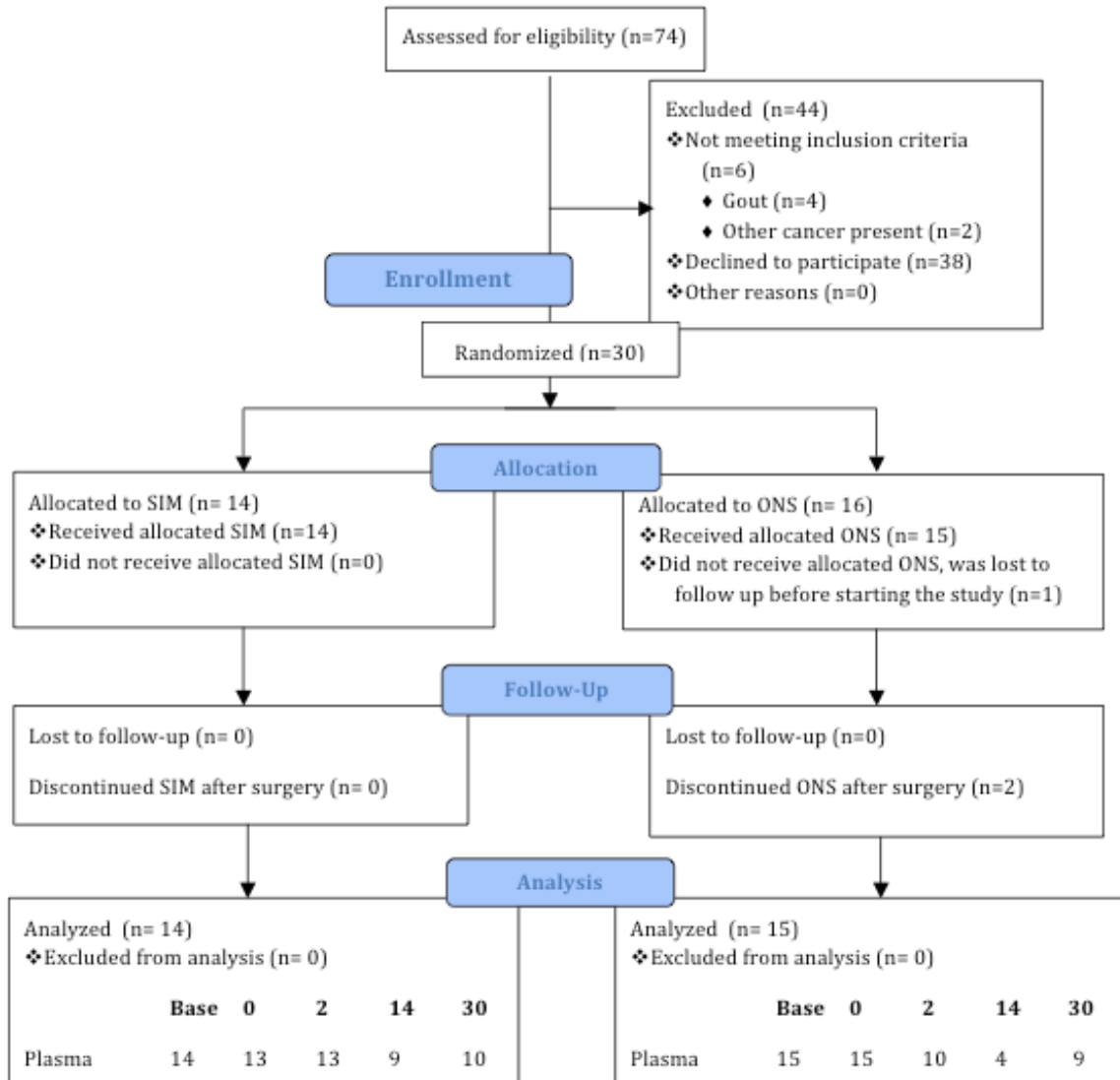


Figure 2- Participant flow chart

Table 3. Baseline characteristics of participants

Characteristic	SIM Group	ONS Group
N	14	15
Age, mean \pm SD	69.6 \pm 7.1	68.1 \pm 8.0
Smoking status, No. (%)		
Current Smoker	4 (28.6%)	1 (6.7%)
Former Smoker	9 (64.3%)	12 (80.0%)
Clinical Stage, No. (%) ¹		
CIS	4 (28.6%)	8 (53.3%)
Ta	1 (7.1%)	2 (13.3%)
Tis	0 (0%)	2 (13.3%)
T1	2 (14.3%)	4 (26.7%)
T2	12 (85.7%)	7 (46.7%)
Charlson Comorbidity Index ²	4.9 \pm 0.8	5.1 \pm 1.6
Neoadjuvent Chemotherapy, No. (%)	8 (57.1%)	4 (26.7%)
BMI*, kg/m ² \pm SD	25.8 \pm 4.0	29.4 \pm 4.7
PG-SGA	6.7 \pm 5.2	5.5 \pm 7.2
Urinary Diversion Type		
Neobladder	3 (21.4%)	6 (60.0%)
Ileal conduit	11 (78.6%)	9 (40.0%)

BMI = Body Mass Index; PG-SGA= Patient Generated Subjective Global Assessment

¹ NCCN Guidelines Version 1.2014 Staging Bladder Cancer (AJCC, 7th Ed., 2010)

² Higher score indicates greater severity of comorbid conditions

**P* = 0.04

Postoperative Complications

Early period

Early complications (first 30 days) occurred in 10 patients (71%) within the SIM group and 11 patients (73%) in the ONS group. The two groups were similar for rates of high-grade complications, type of complications experienced, readmission rates, and SIRS during the early period (Table 4 and Appendix B). When stratified by highest Clavien-Dindo

grade of complication, there were no significant differences seen between the groups during the early phase (Table 5).

Late period

Late complications (31-90 days) occurred in 2 patients (14%) within the SIM group and 6 patients (43%) in the ONS group ($P = 0.21$). Within the ONS group, 50% of participants received non-prophylactic antibiotics compared to 14% in the SIM group ($P = 0.10$). The two groups were statistically similar for rates of high-grade complications and type of complications experienced during the late period (Table 4 and Appendix B). When stratified by highest Clavien-Dindo grade of complication, there were no significant differences seen between the groups during the late phase (Table 5).

Overall, complications occurred in 75.9% of participants in the 90 days following surgery, with no participant deaths. Total length of stay for the SIM and ONS group was similar (6.3 and 6.1, respectively, $P = 0.84$).

Table 4 - Postoperative complications

	SIM Group*	ONS**	P value
Complications, No. (%)			
30 day	10 (71.4%)	11 (73.3%)	1
90 day	2 (14.3%)	6 (42.8%)	0.21
High-Grade Complications¹, No. (%)			
30 day	2 (14.3%)	2 (13.3%)	1
90 day	0 (0%)	2 (14.3%)	0.48
Antibiotic Use², No. (%)			
30 day	5 (35.7%)	9 (60.0%)	0.27
90 day	2 (14.3%)	7 (50.0%)	0.10
Intra-abdominal Infection, No. (%)			
30 day	1 (7.1%)	3 (20.0%)	0.33
90 day	0 (0%)	1 (7.1%)	1
Surgical Site Infection, No. (%)			
30 day	0 (0%)	1 (7.1%)	1
90 day	0 (0%)	0 (0%)	1
Ileus³			
30 day	4 (28.6%)	2 (13.3%)	0.39
90 day	0 (0%)	0 (0%)	1
Length of Stay, days			
	6.3 ± 3.1	6.1 ± 1.9	0.84
SIRS⁴, No. (%)			
	1 (7.1%)	2 (13.3%)	1
Readmission⁴, No. (%)			
	4 (28.6%)	6 (40.0%)	0.70

¹Clavien-Dindo grades III-V

²Non-prophylactic

³Delay in feeding of greater than or equal to five days postoperatively

⁴Systemic inflammatory response syndrome beyond 72 hours after surgery

⁵Within the first 30 days following surgery

*n = 14 for SIM during both time periods

**n = 15 and 14 for 30 day and 90 day time periods, respectively

Table 5- Postoperative complications by Clavien-Dindo grade

	SIM Group	ONS Group
30 day	(n = 14)	(n = 15)
Grade 0	4 (28.6%)	4 (26.7%)
Grade I	1 (7.1%)	0 (0%)
Grade II	7 (50%)	9 (60.0%)
Grade III-a	1 (7.1%)	1 (6.7%)
Grade III-b	0 (0%)	1 (6.7%)
Grade IV-a	0 (0%)	0 (0%)
Grade IV-b	1 (7.1%)	0 (0%)
Grade V	0 (0%)	0 (0%)
90 Day	(n = 14)	(n = 14)
Grade 0	12 (85.7%)	8 (57.1%)
Grade I	0 (0)	0 (0)
Grade II	2 (14.3%)	4 (28.6%)
Grade III-a	0 (0%)	2 (14.3%)
Grade III-b	0 (0)	0 (0)
Grade IV-a	0 (0)	0 (0)
Grade IV-b	0 (0)	0 (0)
Grade V	0 (0)	0 (0)

Dietary Intake

Dietary intake of total energy, protein, arginine, EPA, or DHA was similar between the two groups at each of the time points (Table 6). The ONS group had a significantly lower intake of vitamin A at POD 14 compared to the SIM group ($P = 0.03$).

Table 6- Nutrient intake

	SIM Group, mean \pm SD (n)	ONS Group mean \pm SD (n)
Energy, kcal/d		
Baseline	2699 \pm 876 (14)	2151 \pm 941 (15)
POD 14	2323 \pm 925 (9)	1442 \pm 942 (5)
POD 30	2660 \pm 1263 (10)	2248 \pm 608 (10)
Total Protein, g/d		
Baseline	101 \pm 38 (14)	84 \pm 36 (15)
POD 14	85 \pm 37 (9)	73 \pm 55 (5)
POD 30	97 \pm 45 (10)	88 \pm 29 (10)
Arginine Intake, g/d		
Baseline	5.2 \pm 2.2 (14)	4.5 \pm 2.2 (15)
POD 14	4.4 \pm 2.2 (9)	4.0 \pm 3.4 (5)
POD 30	5.1 \pm 2.4 (10)	4.9 \pm 1.8 (10)
Vitamin A, IU/d		
Baseline	6662 \pm 7179 (14)	11392 \pm 10975 (15)
POD 14*	12653 \pm 11604 (9)	2945 \pm 2356 (5)
POD 30	6183 \pm 7236 (10)	5909 \pm 4746(10)
EPA, g/d		
Baseline	0.01 \pm 0.01 (14)	0.01 \pm 0.01 (15)
POD 14	0.11 \pm 0.30 (9)	0.01 \pm 0.01 (5)
POD 30	0.01 \pm 0.02 (10)	0.02 \pm 0.01 (10)
DHA, g/d		
Baseline	0.04 \pm 0.04 (14)	0.03 \pm 0.03 (15)
POD 14	0.15 \pm 0.31 (9)	0.07 \pm 0.07 (5)
POD 30	0.03 \pm 0.04 (10)	0.05 \pm 0.06 (10)

¹Based on 24-hour dietary recall

*Statistically significant, $P = 0.03$

Amino Acids

Arginine deficiency

At the time of surgery, 8 patients in the SIM group (61%) and 10 in the ONS group (67%) were considered arginine deficient, with no significant difference between groups (OR 1.24, 95% CI 0.20-7.68). Two days following surgery, 11 participants in the SIM group (85%) and 9 in the ONS group (90%) were arginine deficient, with no significant difference between the two groups (OR 1.6, 95% CI 0.07-107.03).

Arginine and infection

The relationship between plasma arginine status and infection rate (using non-prophylactic antibiotic use as the surrogate marker) was investigated looking at arginine as a continuous and categorical (deficient or sufficient) variable. Plasma arginine status at the time of surgery had no effect on antibiotic usage when considering plasma arginine as a continuous (OR 1.01, 95% CI 0.98-1.04) or categorical variable (OR 1.88, 95% CI 0.40-9.66). Similarly, plasma arginine at POD 2 had no effect on antibiotic usage when considering plasma arginine as a continuous (OR 0.99, 95% CI 0.95-1.04) or categorical variable (OR 0.50, 95% CI 0.021-6.06).

Arginine over time

Arginine concentrations were not significantly different between the two groups over time ($P = 0.72$) (Figure 3). Plasma arginine significantly decreased from baseline to POD2 ($P = 0.0003$) and significantly increased from baseline to POD 30 ($P = 0.002$). There was no significant change in plasma arginine from baseline to any time point in the SIM group ($P > 0.05$).

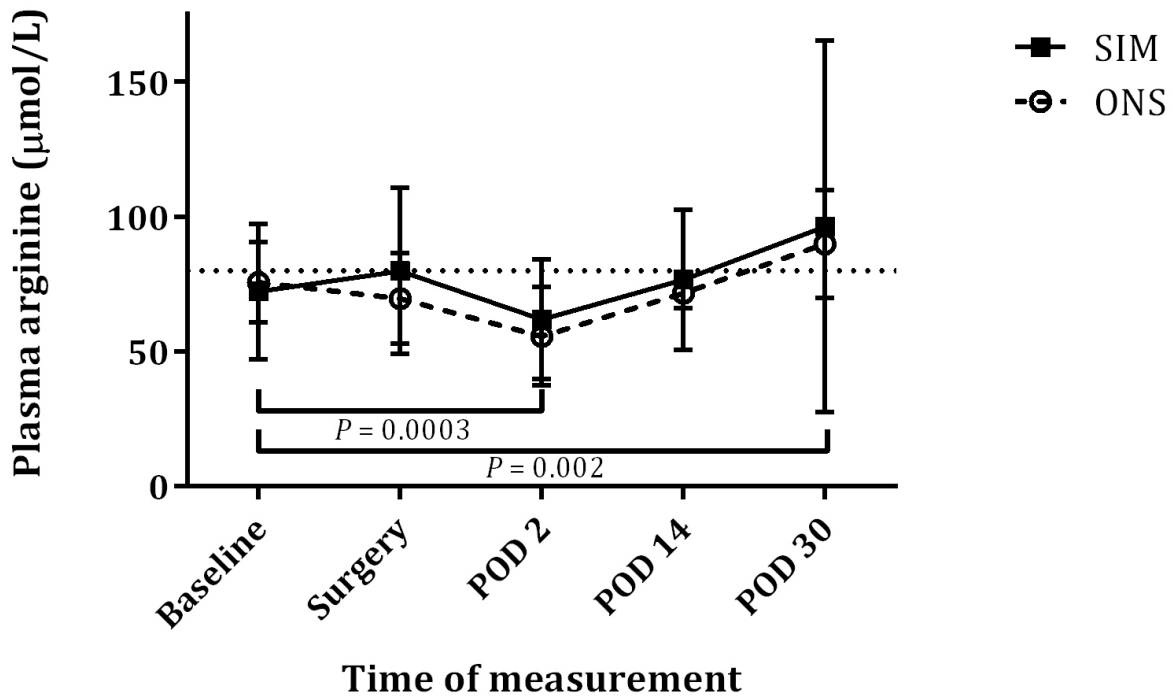


Figure 3- Plasma arginine concentrations over time. There is a significant decrease in plasma arginine concentrations in the ONS group from baseline to POD 2 ($P = 0.003$) and a significant increase from baseline to POD 30 ($P = 0.002$). The dotted line represents the bottom of the reference range for plasma arginine ($80 \mu\text{mol/L}$).

Ornithine

Ornithine concentrations were significantly different between the two groups over time ($P = 0.04$) (Figure 4). At time of surgery time, the SIM group had significantly higher plasma ornithine concentrations compared to the ONS group ($P = 0.001$). There were no significant differences in ornithine concentrations between the two groups at POD 2, 14, and 30.

Within the ONS group, a significant decrease in plasma ornithine was seen from baseline to surgery ($P = 0.01$) and POD 2 ($P = 0.002$). Conversely, a significant increase in plasma ornithine was seen from baseline to surgery in the SIM group ($P = 0.03$).

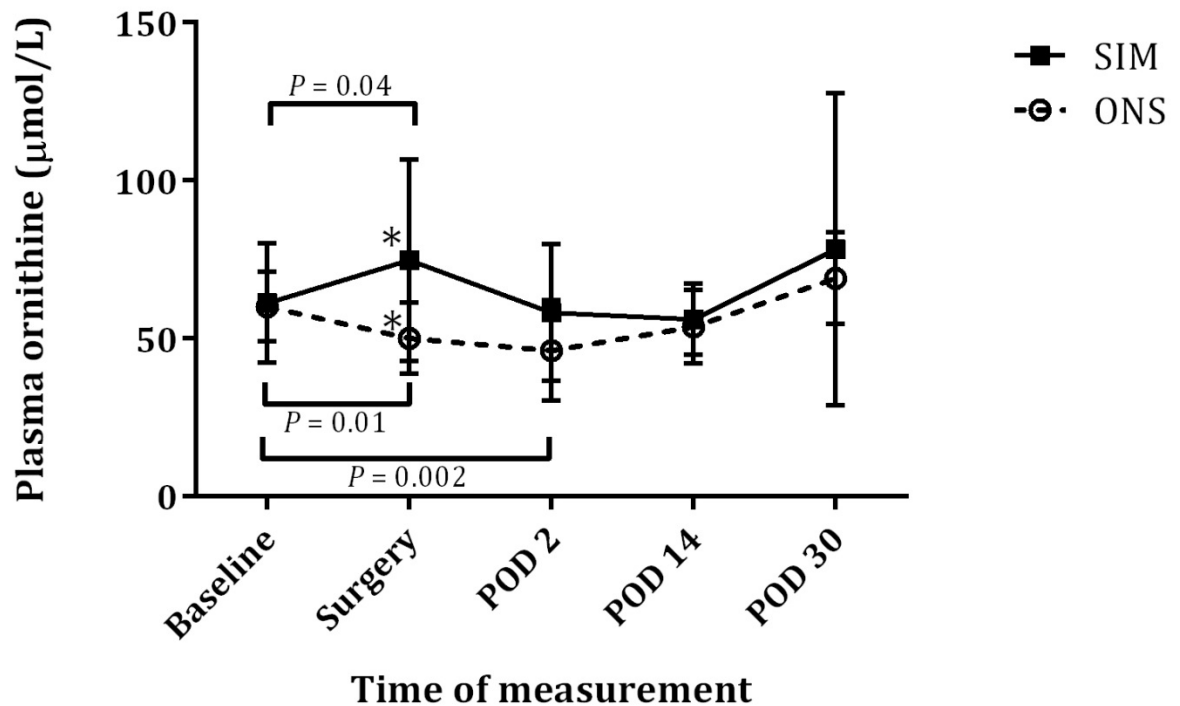


Figure 4- Plasma ornithine concentrations over time. Ornithine concentrations were significantly different between the two groups over time ($P = 0.04$). The two groups had significantly different ornithine levels at surgery ($*P = 0.001$). The control group had a significant decrease in ornithine concentrations from baseline to surgery ($P = 0.01$) and POD 2 ($P = 0.002$). The SIM group had a significant increase in plasma ornithine from baseline to surgery ($P = 0.03$).

Citrulline

Citrulline concentrations were not significantly different between the two groups over time ($P = 0.10$) (Figure 5). Plasma citrulline decreased from baseline to POD 2 in both the ONS ($P = 0.0006$) and SIM ($P < 0.0001$) groups. There were no significant differences in plasma citrulline between baseline and any other time point in either group.

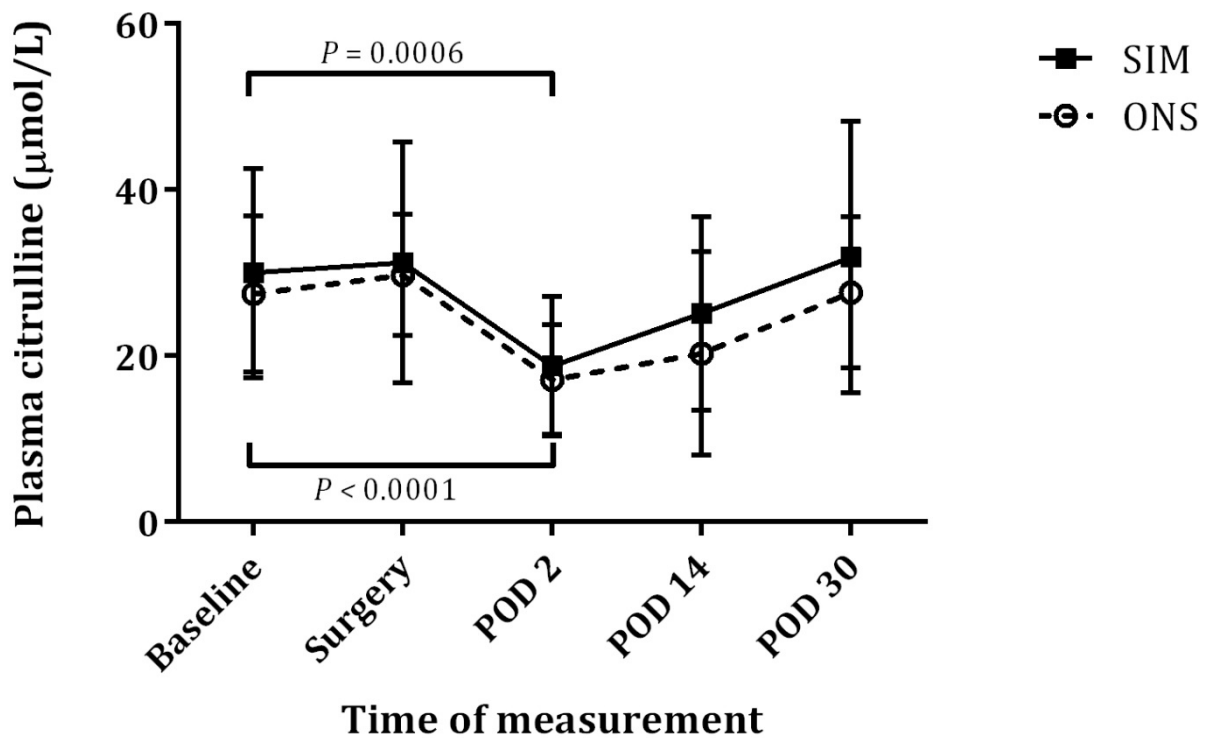


Figure 5- Plasma citrulline concentrations over time. Both groups experienced a significant decrease in plasma citrulline at POD 2 from baseline ($P = 0.0006$ for ONS and $P < 0.0001$ for SIM).

Chapter 5- Discussion

This study investigated the effects of perioperative specialized immunonutrition (SIM) on postoperative complications and plasma amino acid concentrations in bladder cancer patients undergoing radical cystoprostatectomy (RC). Ours was the first placebo-controlled study to date to investigate SIM supplementation in the RC cohort. The findings of this study suggest SIM supplementation alters amino acid metabolism and produces a trend toward decreased postoperative infections.

Postoperative complications

Participants receiving SIM supplementation had a 36% reduction in antibiotic use during the late phase of recovery (Table 4). This reduction suggests SIM supplementation may lead to a lower rate of infection, which was reported previously by Bertrand et al. (11). Arginine is necessary for proper T lymphocyte function (53). Therefore, arginine depletion from surgical trauma may contribute to immunosuppression and susceptibility to infection (12, 54). As our data suggest, the repletion of arginine through SIM supplementation may reduce risk of infection during recovery.

Our study did not find a significant decrease in total complications with SIM supplementation (Table 4), in contrast to the study conducted by Bertrand et al. (11) that found a 37% decrease in complication rate with the use of SIM ($P = 0.008$). However, the inclusion of the iso-caloric/iso-nitrogenous control in our study may have mitigated some of the discrepancies in complication rates seen by Bertrand et al. (11). Previously, high risk of malnutrition has increased complication rates threefold in urological cohorts (23). The average score on the PG-SGA, a tool validated in oncologic populations for assessing physical findings of malnutrition (51), were 6.7 and 5.5 for the SIM and ONS groups, respectively. Based on PG-SGA scoring, a score of >5 suggests the need for nutritional intervention via a dietitian. Therefore, our cohort entered with a risk of malnutrition, suggesting perioperative nutritional support in the form of either SIM or ONS could reduce complications.

Postoperative ileus is a common complication following RC, occurring in 25% of patients in a large contemporary cohort (4). Our study saw a slightly lower rate in ileus among all study participants (21%) with no significant difference between the two groups.

Therefore, it is possible that early feeding, as employed by enhanced recovery after surgery (ERAS) protocols (77), may reduce ileus, regardless of the presence of the immunonutrients. Betrand et al. (11) reported a much lower rate of ileus (7%) with preoperative SIM, but had identified ileus at postoperative day 7, rather than day 5.

The morbidity rate in our study (76%) is higher than that reported in the literature (64%) (4). However, there were no deaths in our cohort compared to the previously reported 90-day mortality rate of 2-7% (4, 38). Furthermore, the average scores for the Charlson comorbidity index of around 5 in both groups also suggest that this cohort may have been at a greater risk for complications than the general RC population. Rohgmann et al. (78) found only 18% of a contemporary European RC cohort scored 3 or higher on the Charlson comorbidity index compared to 100% of our cohort. Furthermore, the authors found a score of 3 or higher increased risk of complications by 93% (78). As a prestigious oncology surgical center, referrals of high-risk patients may also inflate the complication rate seen at our center.

Amino Acids

The plasma amino acid analysis in this study brings a new contribution to the literature regarding SIM use in the RC cohort. This data brings insight into both the typical amino acid metabolism surrounding RC, as well as the shift caused by the supplementation of the immunonutrients.

Arginine

Much of the theory surrounding the efficacy of SIM involves the ability of the immunonutrient supplementation to prevent “arginine deficiency syndrome” (ADS) after

surgery (17). However, our data revealed that prior to surgery, the RC patients already had arginine concentrations below the reference range (Figure 3) (15). Given that the rise of MDSCs and subsequent ADS have been implicated in certain cancers (17, 54, 79), it is possible that residual tumors even after previous resection in our participants may cause this arginine deficiency at baseline. Even with SIM supplementation, arginine levels were not fully replete at the time of surgery. However, SIM supplementation did prevent further depletion of arginine at POD 2 that was seen in the ONS group. The lack of significant change seen in postoperative complications may be related to the inability of SIM to fully replete arginine and restore immune function, suggesting 14g/day of arginine is insufficient in meeting the preoperative needs of RC patients.

Ornithine

Contrary to the expected change in arginine, our data showed that the significant shift in amino acid metabolism from SIM occurred in ornithine concentrations (Figure 4). Ornithine is the product of arginine metabolism by arginase. Therefore, one would surmise that an increase in arginase activity after surgery would cause an increase in ornithine concentration. However, the ONS group experienced a decrease in plasma ornithine at surgery and POD 2. Similarly, a study by Hol et al. (80) reported a decrease in plasma ornithine from the beginning to the end of surgery, a decrease that persisted for 24 hours. Low levels of ornithine have also been seen following trauma (18, 81), even in the presence of increased arginase 1 activity (81). The significant decrease in ornithine seen at three hours post-incision and POD 2 in the ONS group is consistent with these findings and is therefore not indicative of a decrease in arginase activity, but likely a lack of available substrate, arginine.

Conversely, a significant increase in plasma ornithine from baseline to surgery occurred in the SIM group. Previously, Tsuei et al. (18) found that arginine supplementation following trauma is metabolized to ornithine. In fact, plasma ornithine concentrations increased in response to the intervention six days prior to an increase in plasma arginine (18). Therefore, while SIM produced no significant change in plasma arginine levels in our study, the change in ornithine does reflect a greater availability of substrate for arginase activity.

The implications of an increase in plasma ornithine concentrations during surgery produced by SIM are yet to be fully elucidated. However, considering ornithine's metabolic pathways, such increase may confer some benefit. Ornithine is metabolized into by ornithine decarboxylase and ornithine aminotransferase to proline and polyamines (Figure 1), which may contribute to wound healing through collagen formation and cell proliferation (17). In comparing the amino acid differences surrounding major and minor surgeries, Hol et al. (80) reported ornithine levels were significantly lower following a major surgery compared to a minor surgery, while the arginine levels did not differ between groups. The authors logically concluded that the difference in ornithine concentrations could not be related to arginase activity but rather ornithine usage for wound healing (80). Therefore, SIM supplementation may lead to better wound healing by increasing ornithine availability for proline and polyamine production; however, our study neither tracked nor reported clinical evidence to this effect. Furthermore, caution should be used when interpreting the increase in ornithine availability at surgery, considering no changes in infection rates were seen in the supplemented trauma patients (18), and limiting ornithine decarboxylase activity to decrease cellular proliferation has been

suggested for cancer therapy (82). Nevertheless, the role of arginine supplementation for wound healing through proline and polyamine production has been suggested previously and may enhance surgical recovery (83).

Citrulline

Regarding plasma citrulline, a product with nitric oxide (NO) of arginine metabolism and a precursor for endogenous production (Figure 1), both groups saw a significant decrease at POD 2 (Figure 5). Due to the dual role of citrulline in arginine metabolism, interpretation of this data on its own is difficult. However, a similar decrease in plasma citrulline after surgery was previously reported along with a decrease in NO metabolites (80). Therefore, the increase in arginase 1 activity may limit arginine availability for nitric oxid synthase to produce citrulline and NO following surgery. Due to the importance of NO in vasodilation and pathogen defense, limited production may contribute to poor outcomes following RC. This is consistent with previous theories regarding the metabolic consequences of ADS (17), suggesting the SIM intervention in this study was insufficient to meet all the metabolic needs surrounding RC.

Strengths, Limitations, and Future Research

This study suggests SIM may reduce postoperative infections and induce a shift in amino acid metabolism in RC patients, adding to the literature regarding this cohort. However, the study is not without limitations. Most notably, the small sample size made statistical significance difficult to achieve. Therefore, it is possible that the trend toward a decrease in postoperative complications seen with SIM use could become statistically significant in a larger sample. While conducting the study within one center allowed for a

greater amount of control over protocol and procedures, a multi-center study is needed for greater external validity. Additionally, a double-blinded design would maximally reduce bias.

Primary to this study's many strengths is its contribution as the first randomized-controlled trial for SIM in the RC population. The iso-caloric/iso-nitrogenous ONS allowed for a stronger investigation of the immunonutrients over calories and protein alone. Additionally, this was the first trial to supplement SIM for the entire perioperative period. In terms of grading the complications, the use of a standardized grading system (Clavien-Dindo) decreased subjectivity for determining severity level. Furthermore, the use of non-prophylactic antibiotics as surrogate markers for infection reduced ambiguity in tracking, though prescription without a positive culture could have inflated the infection rate. Finally, this study combined both clinical outcome data along with plasma amino acids in order to contribute to the literature regarding the etiology and clinical relevance of SIM in the RC population.

While many critical components were included in study outcomes, arginase 1 activity was not measured and could be useful in the future for better understanding the biological impact of SIM. Adding more time points for amino acid analysis, such as just prior to surgery, could help differentiate the impact from supplementation and surgery. Employing a more sophisticated technique for tracking amino acids, such as an isotope tracker, could expand the understanding of amino acid metabolism (84). Ultimately, larger, double-blinded trials are still needed.

Conclusion

In conclusion, the perioperative supplementation of SIM in RC patients induces a shift in metabolism of amino acids that are related to immune function and wound healing. Even prior to surgery, RC patients have low plasma arginine concentrations. Though SIM supplementation was unable to fully replete arginine status by surgery, the supplementation prevented a further depletion in plasma arginine following surgery. Preserving arginine concentration after surgery by the supplementation of SIM may prevent the decline in immune function that often follows trauma. Our data present evidence to this effect in the trend toward lower infection rates seen with SIM supplementation. However, a greater amount of arginine may be necessary to fully replete this population and optimize immune function following surgery. The greatest change in amino acid metabolism was reflected not in plasma arginine concentrations, however, but in plasma ornithine. The increased concentrations of ornithine during surgery in the SIM group suggest that the supplemented arginine is metabolized to ornithine. A greater availability of ornithine during surgery recovery may lead to greater proline and polyamine production for enhanced wound healing. However, more research is needed to determine the implications of the amino acid changes on recovery outcomes and confirm the benefits of perioperative SIM supplementation in the RC cohort.

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Appendix A: Systemic Inflammatory Response Syndrome Criteria

Systemic inflammatory response syndrome (SIRS) criteria (43)

Category ¹	Criteria
Temperature	>38° C or <36° C
Heart rate	>90 beats/min
Respiration rate	>20 breaths/min or PaCO ₂ <4.3 kPa
White blood cell count	>12,000 cells/mm ³ or <4000 cells/mm ³ or >10% bands present

¹Two or more of the criteria must be present to classify for SIRS

Appendix B: Postoperative Complications by Type

Postoperative complications by type

	SIM Group	ONS Group
Dehiscence		
30 day	0 (0%)	1 (6.7%)
90 day	0 (0%)	1 (6.7%)
Return to Operating Room		
30 day	0 (0%)	1 (6.7%)
90 day	0 (0%)	0 (0%)
Pulmonary Embolus		
30 day	2 (14.3%)	0 (0%)
90 day	0 (0%)	0 (0%)
Pneumonia		
30 day	0 (0%)	1 (6.7%)
90 day	0 (0%)	0 (0%)
Urinary Tract Infection		
30 day	0 (0%)	1 (6.7%)
90 day	2 (14.3%)	2 (14.3%)
Pyelonephritis ¹		
30 day	0 (0%)	1 (6.7%)
90 day	0 (0%)	2 (14.3%)
Sepsis		
30 day	1 (7.1%)	1 (6.7%)
90 day	0 (0%)	0 (0%)

¹Pyelonephritis, a more severe form of urinary tract infection, was assessed separately as an outcome, but counted and graded only once as a urinary tract infection

P > 0.05 for all categories