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Immunonutrition before esophagectomy: Impact on immune surveillance mechanisms

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Abstract

Preoperative oral immunonutrition was demonstrated to improve immune response and to decrease the infection rate in patients with cancer. This study aimed to assess how immunonutrition could influence the immune cell response in the mucosal microenvironment of esophageal adenocarcinoma. Therefore, A prospective cohort of consecutive patients undergoing esophagectomy for esophageal adenocarcinoma was enrolled. A subgroup of them was given preoperative oral immunonutrition with Oral ImPact® and was compared to those who received no preoperative supplementation. Mucosal samples from healthy esophagus were obtained at esophagectomy. Histology, immunohistochemistry, gene expression analysis, and cytofluorimetry were performed. Markers of activation of antigen-presenting cells (CD80, CD86, and HLA-I), innate immunity (TLR4 and MyD88), and cytotoxic lymphocyte infiltration and activation (CD8, CD38, CD69, and CD107) were measured. In all, 50 patients received preoperative Oral ImPact® and 129 patients received no nutritional support. CD80, CD86, MyD88, and CD69 messenger RNA expression was significantly increased in patients receiving immunonutrition compared to controls. In the subgroup of patients with stages I–II cancer, the rate of epithelial cells expressing CD80 and HLA-ABC was significantly higher in those receiving immunonutrition compared to controls as well as CD8+ CD28+ cell rate. Immunonutrition administration before surgery was significantly associated to increased degranulating CD8 and natural killer cells (CD107+) infiltrating the healthy esophageal mucosa. All the comparisons were adjusted for cancer stage and preoperative therapy. In conclusion, in healthy esophageal mucosa of patients undergoing esophagectomy, a 5-day course of immunonutrition enhances expression of antigen-presenting cells activity and increased CD8+ T cell activation and degranulating activity. Further studies are warranted to understand the clinical implication in terms of cancer recurrence.

Keywords

Immunonutrition, esophageal adenocarcinoma, immunosurveillance, CD80, CD8

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Background

Esophageal adenocarcinoma is the fastest rising upper gastrointestinal malignancy in the Western world and its outcome remains poor.[1] Combined modality treatment protocols such as neoadjuvant radiation and/or chemotherapy followed by surgery represent the current treatment option.[2,3] However, only patients with a complete pathologic response to neoadjuvant therapy seem to experience a significantly better chance of survival.[4,5] Nevertheless, esophagectomy remains the standard treatment for patients presenting with resectable esophageal cancer but it is associated with a high risk of serious complications.[6–11]

Immunonutrition has been reported to improve the immune status of perioperative cancer patients. Preoperative oral combination of arginine and n-fatty acids (immunonutrition) was demonstrated to improve immune response and to decrease the infection rate in patients with colorectal cancer.[12] In particular, it appeared to be effective for preventing surgical site infections.[13] Moreover, in patients with head and neck and esophageal cancer undergoing radio-chemotherapy, immunonutrition was associated with a significant gain in total body weight and with the maintenance of functional capacity.[14] However, a 2013 meta-analysis of six randomized controlled trials that compared the clinical benefits of immunonutrition after surgery for esophageal cancer failed to demonstrate consistent improvements in postoperative clinical course, complications, length of hospital stay, and inflammatory marker levels.[15] Nevertheless, in 2014, a further study showed that patients receiving preoperative immunonutrition experienced significantly fewer postoperative infections compared with the standard group and reported a significantly higher 6-month survival rate.[16]

All these studies focused on the immediate postoperative effects of immunonutrition, and so far, no data are available on its impact on the immune microenvironment of esophageal cancer. In fact, mucosal immune surveillance mechanisms might play a key role in local recurrence after esophagectomy, as in the recurrence of colorectal cancer.[17,18] In a recent study, in colorectal cancer patients who were given different nutrition regimens in the 7-day preoperative period, a marked increase in CD8(+) cells was observed within the tumor in those who had received preoperative immunonutrition.[19] Therefore, the current challenge is to analyze whether immunonutrition influences the immune status and modulates acquired and innate immune responses to cancer to improve patient outcomes.[20] Thus, this study aimed to assess whether immunonutrition enriched with arginine, omega-3 fatty acids (eicosapentaenoic acid and docosahexaenoic acid), and nucleotides could influence the immune cell response in the mucosal immune microenvironment of esophageal adenocarcinoma patients.

Patients and methods

Study design

This study is based on a secondary analysis of the patients included in the MICCE1 (Microambiente Immunitario Carcinoma del Colonretto e dell'Esofago—Step 1) project. The MICCE1 project aimed to evaluate the immunological microenvironment of esophageal and colorectal cancer.[21,22] It included a prospective cohort of 179 consecutive patients who underwent esophagectomy for esophageal adenocarcinoma. The MICCE1 project is an observational prospective project and no experimental interventions were associated with the MICCE1 design. Among these patients, 50 had also been enrolled in the Quality of Life After Esophagectomy for Cancer—Step 1 (QOLEC; NCT01738620; http://www.clinicaltrials.gov) trial[23] and received preoperative oral immunonutrition with Oral Impact® before surgery as standard care. Therefore, these 50 patients who received preoperative oral immunonutrition were compared with the remaining 129 patients who received no preoperative supplementation. The aim of this study was to evaluate whether immunonutrition could influence the immune cell response in the mucosal immune microenvironment of esophageal adenocarcinoma patients. The study was performed according to the principles of the Declaration of Helsinki, all participants provided informed consent, and Institutional Review Board (IRB) approval (Veneto Institute of Oncology, Padova, Italy) was obtained for both protocols (MICCE1 and QOLEC1). For each patient, appropriate clinical and follow-up data were collected. Diagnosis was confirmed by clinical, radiological, and histological parameters.

Mucosal samples from healthy esophagus were obtained at esophagectomy. For each sample, histology, immunohistochemistry, gene expression analysis (quantitative reverse transcription polymerase chain reaction (RT-PCR)), and cytofluorimetry were performed. Markers of activation of antigen-presenting cells (APCs; CD80 and CD86 expression), innate immunity (TLR4 and MyD88 expression), and cytotoxic lymphocyte infiltration, activation, and degranulation (CD8, CD38, CD69, and CD107) were measured and compared between the two groups. Immunological systemic status was assessed with full blood count as well as the number of circulating lymphocyte before and after esophagectomy.

Immunonutrition scheme

Oral Impact® (Nestlé Health Science, Epalinges, Switzerland) is a powdered oral feed that provides 1.0 kcal/mL when reconstituted with water. Nutritionally complete Food for Special Medical Purposes contains omega-3 fatty acids, arginine, nucleotides, and soluble fiber. Before

surgery, the immunonutrition group consumed 750 mL (three packs)/day of Oral Impact® for five consecutive days.

Histology

Sections (3 μm) from formalin-fixed and paraffin-embedded human specimens were stained with hematoxylin–eosin. Histological inflammation was quantified and classified by a pathologist (M.F.), unaware of the experimental arm, using the Padua classification of gastric epithelial neoplasia, according to World Health Organization (WHO, 2010) classification.[24,25]

RT-PCR of esophageal tissue

Total RNA from intestinal tissue was extracted using the SV Total RNA Isolation System (Promega) according to the manufacturer's instructions. Complementary DNA (cDNA) synthesis was performed using the Applied Biosystems cDNA Synthesis kit according to the manufacturer's directions. Specific messenger RNA (mRNA) transcripts were quantified with SYBR Green PCR Master Mix in an ABI PRISM 7000 Sequence Detection System (Applied Biosystems). The expression of the target molecule was normalized to the expression of the Beta Actin housekeeping gene. The primers for human CD80, CD86, TLR4, MyD88, CD38, and CD69 were used at RT-PCR conditions described by the producer. CD80 primers were as follows: FW, CTCACTTCTGTTCAGGTGTTATCCA; RV, TCCTTTTGCCAGTAGATGCGA. The primers for human Beta Actin were as follows: FW, CTGGACTTCGAGCAA GAGATG; RV, AGTTGAAGGTAGTTTCGTGGATG

Flow cytometry

Single-cell suspensions were obtained by enzymatic digestion of mucosal specimens and subjected to flow cytometry to determine the proportion of epithelial cells (Cytokeratin, CK+) acting as APCs (expressing CD80, HLA-ABC) and the proportion of activated CD8+ T cells (positive for CD28 and CD38). Esophageal mucosa was stripped from the muscularis mucosa, cut into strips, and freed of mucus by a 30-min wash in Hank's balanced salt solution (HBSS) containing 10 mM dithiothreitol (DTT; AppliChem). Intestinal epithelial cells (IEC) were isolated with a 30-min incubation of the intestinal mucosa in HBSS containing 1 mM ethylenediaminetetraacetic acid (EDTA; Sigma-Aldrich). For lamina propria mononuclear cells (LPMCs), stripped mucosa, freed of mucus and IEC, was digested with 1 mg/mL collagenase and DNase (Sigma-Aldrich) for 30 min at 37°C. The resulting crude cell suspensions were purified using a Ficoll-Hypaque Plus gradient (GE Healthcare), and the preparations were preferentially enriched for LPMC, washed, and collected. Flow cytometric analysis was performed using a FACSCalibur based on CellQuest software (Becton Dickinson). The antibodies used are summarized in Supplementary Table 1.

Immunohistochemistry

Immunohistochemical (IHC) analyses were performed using standard procedures, and the resulting sections were evaluated by a single pathologist in a blinded fashion (M.F.). Immunocomplexes were detected using an avidin–biotin–peroxidase conjugate and 3,3′-diaminobenzidine tetrahydrochloride chromogen as a substrate (ABC Kit; Vector Laboratories and DAB kit; Dako). IHC staining was performed using a monoclonal rabbit anti-CD80 antibody (EP1155Y; Abcam). The expression of CD8 and the leukocyte degranulation markers CD107 were quantitatively measured (number of positive cells for high power field at 40×). The number of 4× fields with moderate/severe infiltration per intestinal surface was obtained and considered a parameter of the immune response to early epithelial mutations. The antibodies used are summarized in Supplementary Table 1.

Statistical analysis

Statistical analysis was performed using R 3.3.2 software (R Foundation for Statistical Computing, Vienna, Austria).[26] All tests were two-sided and a p value below 0.05 was considered statistically significant.

Among patient characteristics, continuous data were expressed as median and interquartile range (IQR) and categorical data as number and percentage. Continuous data were compared between patients receiving and those not receiving preoperative immunonutrition using Mann–Whitney test and categorical data with Fisher's exact test.

Generalized linear models were estimated to assess the effect of preoperative immunonutrition on gene expression levels (CD80, CD86, TRL4, MyD88, CD69, and CD38), on cytotoxic lymphocyte infiltration (CD8+) and activation (CD107+), and on blood circulating lymphocytes (at preoperative assessment and at postoperative day 1). The models included neoadjuvant therapy and tumor stage as clinically relevant confounders and estimated the effect of preoperative immunonutrition as mean differences (MDs) with 95% confidence intervals (95% CIs).

In stage I–II patients, generalized linear models were estimated to assess the effect of preoperative immunonutrition on epithelial cell activity as APC and cytotoxic T lymphocyte activity. The models included neoadjuvant therapy as clinically relevant confounder and estimated the effect of preoperative immunonutrition as MDs with 95% CI.

A Cox regression model was estimated to assess the effect of preoperative immunonutrition on overall survival,

Table 1. Patient characteristics.

	Immunonutrition group (n = 50)	Control group (n = 129)	p value[a]
Demographic data			
Age (years): median (IQR)	63 (56–67)	62 (54–71)	0.93
Sex			0.60
Male	46 (92.0%)	113 (87.6%)	
Female	4 (8.0%)	16 (12.4%)	
Cancer site			
Lower thoracic esophagus	11 (22.0)	17 (13.2)	0.17
Esophago gastric junction	39 (78.0)	112 (86.8)	
Surgical intervention			
Ivor Lewis esophagectomy	50 (100%)	129 (100%)	Not applicable
Neoadjuvant therapy (CT/RT)			
Yes	42 (84.0%)	93 (72.1%)	0.12
No	8 (16.0%)	36 (27.9%)	
Response to neoadjuvant therapy			
Mandard 1	12 (28.6)	15 (16.1)	0.11
Mandard 2–5	30 (71.4)	78 (83.9)	
Pathological N status			
N0	27 (54.0)	71 (55.0)	0.99
N+	23 (46.0)	58 (45.0)	
Cancer pathological stage			
Stages 0–I	13 (26.0)	21 (16.3)	0.34
Stages I–II	21 (42.0)	59 (45.7)	
Stages III–IV	16 (32.0)	49 (38.0)	

IQR: interquartile range; CT/RT: chemotherapy/radiotherapy.
[a]Age was compared between the two groups using Mann–Whitney test, while the categorical variables were compared between the two groups using Fisher's test.

adjusting for neoadjuvant therapy and tumor stage as clinically relevant confounders. A Cox regression model was estimated to assess the effect of preoperative immunonutrition on disease-free survival, adjusting for neoadjuvant therapy and tumor stage as clinically relevant confounders. Survival estimates from these two models were plotted in survival curves for patients receiving and those not receiving preoperative immunonutrition. Overall survival was calculated from date of surgery to date of death, and alive patients were censored at the last date of confirmed alive follow-up. Disease-free survival was calculated from the date of surgery to the date of recurrence or date of death, and patients were censored on the last date of alive and disease-free follow-up.

Results

Patient characteristics

In all, 50 patients received Oral Impact® as preoperative care (case group), and 129 patients received no nutritional support during the preoperative phase (control group). They were 159 males and 20 females. All of them had Ivor Lewis esophagectomy. Patient characteristics are outlined in Table 1.

APC activity, innate immunity, and lymphocyte activation

Gene expression levels (RT-PCR) of APC activity, innate immunity, and lymphocyte activation markers are shown in Figure 1. Patients receiving immunonutrition before surgery showed higher CD80 (MD = 0.0016, 95% CI = 0.0003 to 0.003; p = 0.02), CD86 (MD = 0.02, 95% CI = 0.006 to 0.04; p = 0.007), MyD88 (MD = 0.02, 95% CI = 0.003 to 0.03; p = 0.02), and CD69 (MD = 0.06, 95% CI = 0.02 to 0.10; p = 0.003) mRNA relative levels than patients not receiving immunonutrition. TRL4 and CD38 mRNA relative levels were similar in the two groups (p = 0.13 and p = 0.55, respectively). All analyses were adjusted for neoadjuvant therapy and tumor stage.

Epithelial cells acting as APC and CD8 T cell activation

Cytofluorimetry analysis was stratified for cancer stage and adjusted for neoadjuvant therapy. The results obtained in stages I–II patients are shown in Figure 2. In this subgroup, APC activity in esophageal epithelial cells was enhanced in patients receiving immunonutrition (CK+ CD80+ cell rate: MD = 15.3, 95% CI = 5.3 to

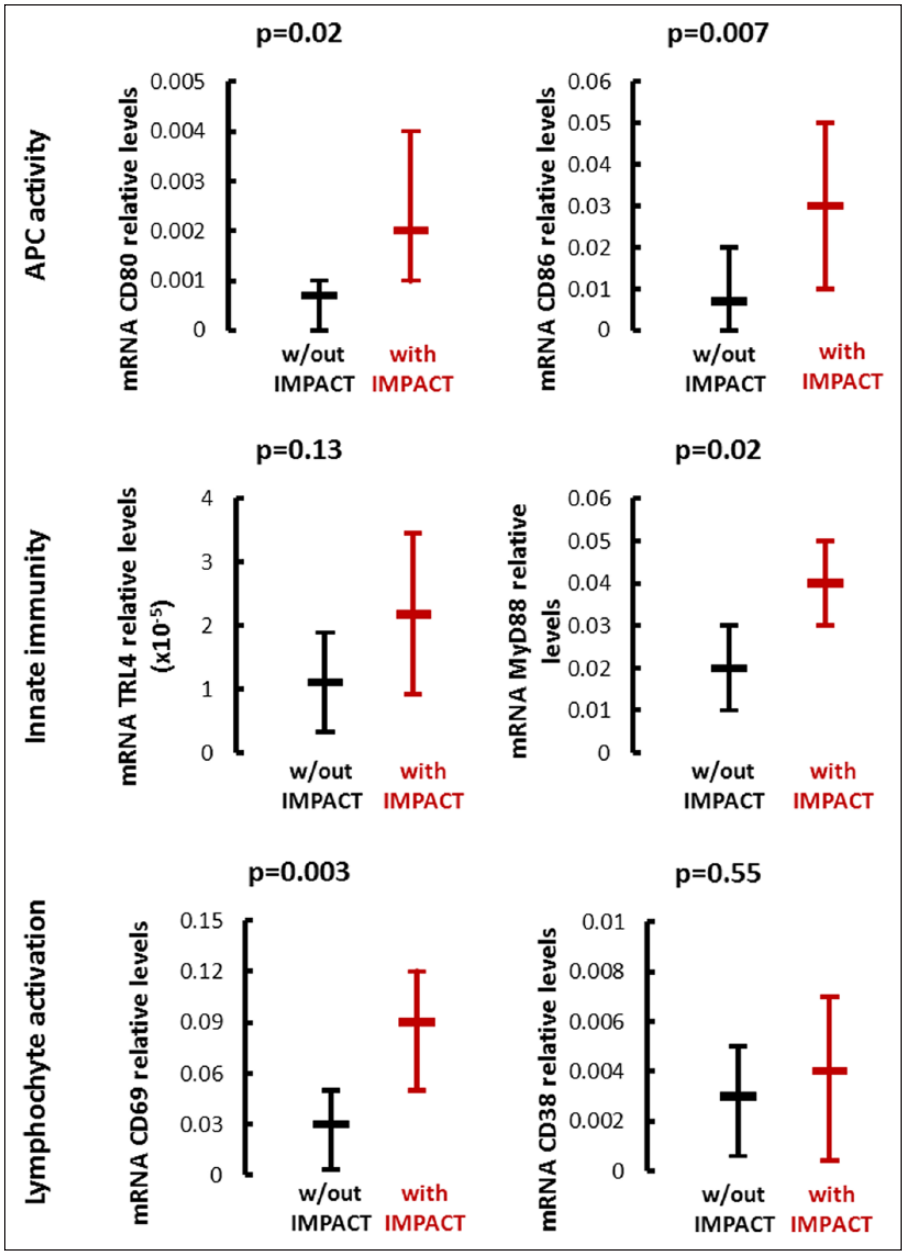

Figure 1. Gene expression levels (RT-PCR) of APC activity, innate immunity, and lymphocyte activation markers in patients receiving and in those not receiving preoperative immunonutrition supplementation (IMPACT): mean (95% CI), adjusted for neoadjuvant therapy, and tumor stage.

25.3; p = 0.003; CK+ HLA+ cell rate: MD = 23.5, 95% CI = 9.4 to 37.6; p = 0.001), adjusting for neoadjuvant therapy. Similarly, in this subgroup, patients receiving immunonutrition before surgery showed higher cytotoxic T cell activation expressed as CD8+ CD28+ cell rate (MD = 2.4, 95% CI = 0.2 to 4.6; p = 0.03) but not CD8+/CD38+ cell rate (MD = –1.6, 95% CI = –6.4 to 3.2; p = 0.53), adjusting for neoadjuvant therapy. In patients with more advanced cancer, no statistically significant difference was observed.

CD8 infiltration and CD8 and natural killer cell activation

Immunohistochemistry analysis of the effect of immunonutrition on CD8 infiltration and on degranulating activity of CD8 and natural killer (NK) cells is shown in Figure 3. Patients receiving immunonutrition before surgery showed higher number of CD107+ cells (MD = 18, 95% CI = 1 to 34; p = 0.04) but not CD8+ cells (MD = 20, 95% CI = –16 to 25; p = 0.68), adjusting for neoadjuvant therapy and tumor stage.

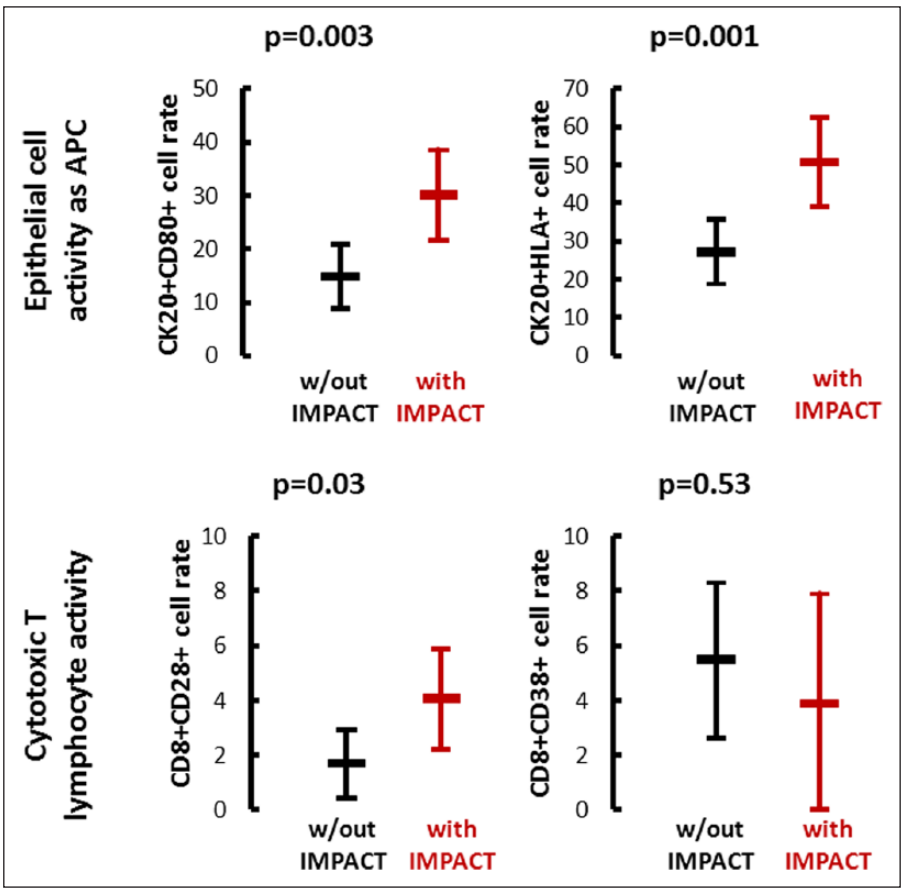

Figure 2. Cytofluorimetry analysis (epithelial cell activity as APC and cytotoxic T lymphocyte activity) in stages I–II patients receiving and in those not receiving preoperative immunonutrition supplementation (IMPACT): mean (95% CI), adjusted for neoadjuvant therapy.

Blood circulating lymphocytes

Effect of immunonutrition on blood circulating lymphocytes is shown in Figure 4. Blood circulating lymphocytes at preoperative assessment was similar between patients receiving and those not receiving immunonutrition before surgery (MD = 0.11, 95% CI = –0.20 to 0.42; p = 0.49). However, patients receiving immunonutrition before surgery showed higher blood circulating lymphocytes than patients not receiving immunonutrition (MD = 0.15, 95% CI = 0.03 to 0.26; p = 0.01). All analyses were adjusted for neoadjuvant therapy and tumor stage.

Long-term effects

Receiving immunonutrition before surgery had no effect on overall survival (hazard ratio (HR) = 1.17, 95% CI = 0.37 to 3.65; p = 0.79) and disease-free survival (HR = 1.20, 95% CI = 0.62 to 2.34; p = 0.59), adjusting for neoadjuvant therapy and tumor stage (Supplementary Figure 1).

Conclusion

Esophageal adenocarcinoma is the fastest rising upper gastrointestinal malignancy in the Western world and its outcome remains poor.[1] In a recent, large European cohort of stage II and III esophago-gastric junction and esophageal adenocarcinoma, the 3-year overall survival rate was 55% and a 3-year disease-free survival was 50% after multimodal therapy.[27] Thus, after definitive treatment of esophageal cancer, patients are still at high risk for recurrence. A recent, large American study reported that recurrence occurred more frequently in the first year after surgery and they were distant in 55% of cases, locoregional in 28%, or both in 17%.[28] Mucosal immune surveillance mechanisms might play a role in local recurrence after esophagectomy as in colorectal cancer recurrence.[17,18] A recent study demonstrated that in colorectal cancer, preoperative immunonutrition was associated with a marked increase of CD8+ T cells within the tumor.[19] Thus, this study aimed to assess whether pre-surgical immunonutrition could influence the immune microenvironment in healthy esophageal mucosa. We focused on healthy esophageal mucosa because this would be the scenario of a potential local recurrence.

In our series, patients who received immunonutrition before surgery showed an increase in innate immunity markers and APC functions in their healthy esophageal mucosa as indicated by their CD80, CD86, and MyD88

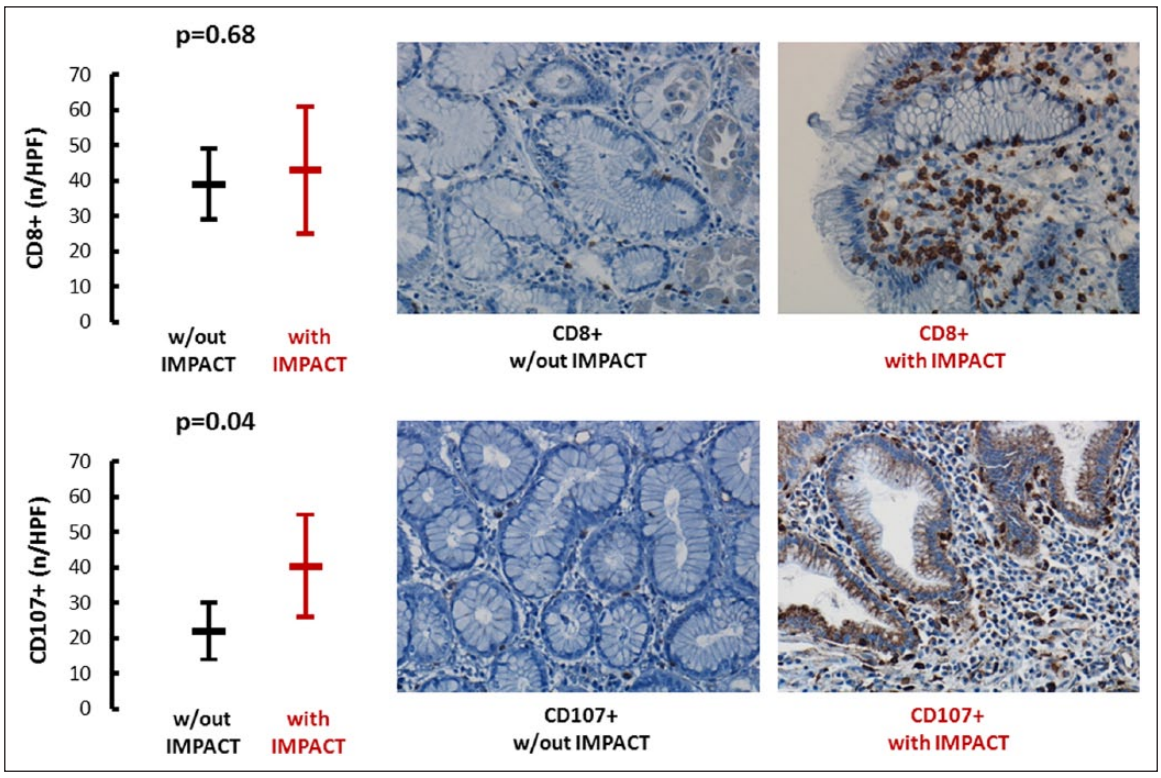

Figure 3. Effect of immunonutrition on cytotoxic lymphocyte infiltration (CD8+) and activation (CD107+; 40× magnification) in patients receiving and in those not receiving preoperative immunonutrition supplementation (IMPACT): mean (95% CI), adjusted for neoadjuvant therapy and tumor stage.

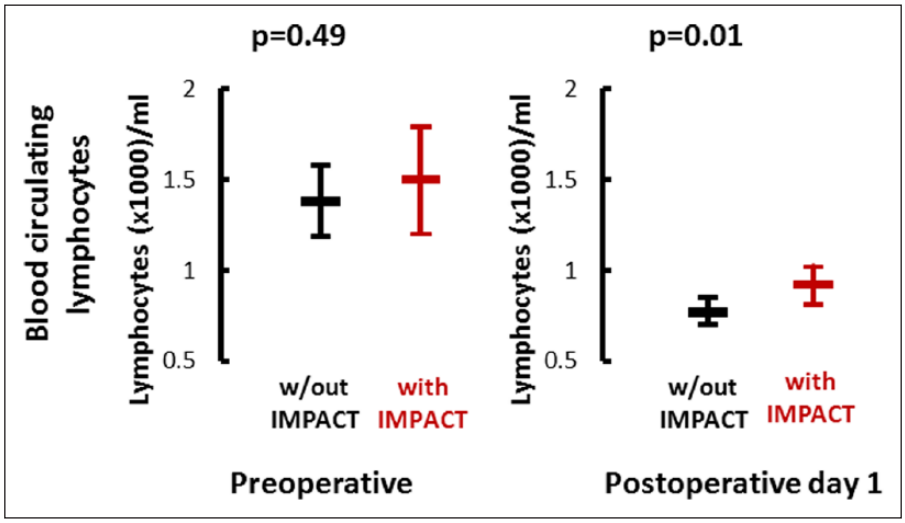

Figure 4. Effect of immunonutrition on blood circulating lymphocytes at preoperative assessment and postoperative day 1 in patients receiving and in those not receiving preoperative immunonutrition supplementation (IMPACT): mean (95% CI), adjusted for neoadjuvant therapy and tumor stage.

mRNA levels compared to control group. Moreover, in the subgroup of patients with stage I and II cancer, a significant higher rate of epithelial cells acting as APC (thus expressing CD80 and HLA-ABC) was observed in patients who received preoperative nutrition compared to control group. Thus, following immunonutrition, esophageal epithelial cells seem to improve their ability to act as APCs, probably improving the immune surveillance mechanisms.[29] On the contrary, in a recent randomized controlled trial, randomly allocating patients undergoing subtotal

esophagectomy and total gastrectomy to receive an O-3FA enteral immunoenhancing diet or standard enteral nutrition, no differences between the groups in HLA-DR expression in either monocytes or activated T lymphocytes were observed.[30] The contrasting results obtained may be due to the different composition of the immunonutrition protocols and to the different cell populations taken in exam. In our opinion, the key role of epithelial cells in initiating the digestive tract immune surveillance mechanisms against cancer[29] makes them a primary target of immune enhancing dietary protocols.

In our series, the number of CD8+ T lymphocytes infiltrating the healthy esophageal mucosa did not change in patients receiving immunonutrition. However, the expression of T cell activation marker CD69 and the number of degranulating CD8 and NK cells infiltrating the esophageal mucosa resulted significantly increased in patients who were given immunonutrition before surgery compared to control group. In a recent study in colorectal cancer patients, a marked increase in CD8+ T cells was observed within the tumor in those who had received preoperative immunonutrition.[19] In several types of neoplasm, CD8+ tumor infiltrating lymphocytes are associated with a more favorable prognosis.[17,18,31] In fact, in our patients with early esophageal cancer, activated (CD28+) CD8+ T lymphocyte rate resulted similar in control patients and patients who had received preoperative immunonutrition. All these data suggest that in healthy esophageal mucosa, immunonutrition enhanced CD8+ T cell activation and T and NK cell degranulation (in particular in early cancer stage) but it did not increase the whole number of cytotoxic T lymphocytes in the esophageal mucosa. This might be due to the short duration of the preoperative immunonutrition that might have prevented their full activation. The same reason might explain why no differences in terms of disease-free survival or overall survival between patients who received immunonutrition and control group were observed. The short duration (five consecutive days) of immunonutrition might have prevented a fully efficient epithelial cell–T lymphocyte interaction, and thus, a long-lasting effect on prevention of recurrence.

Finally, blood circulating lymphocyte concentration significantly decreased at day 1 after surgery but their level was significantly higher in patients who received immunonutrition before surgery compared to control group. In a recent French study, immunonutrition was associated with the maintenance of CD4(+)/CD8(+) T lymphocyte count ratio.[32] Therefore, immunonutrition may be helpful in restoring the lymphocyte systemic pools and in maintaining their correct balance that is associated to a reduced postoperative complication rate.[33]

This study has some limitations. First, it includes a relatively small sample size of patients who received immunonutrition with respect to those who did not receive immunonutrition. Although this aspect may have introduced some bias in the results, surgical treatment was homogeneous in all patients and the two groups were similar in terms of patient characteristics and cancer features. Second, this study was based on a secondary analysis of existing data from MICCE1 and QOLEC studies, thus the available data were clearly not collected to address this particular research question. However, the usual disadvantages of such approach were minimized because no important third variables were unavailable for the analysis and all data were collected and analyzed by the same research team.

In conclusion, in healthy esophageal mucosa of patients undergoing esophagectomy, a 5-day course of immunonutrition enhances expression of CD80 molecule on the surface of APCs and increases CD8+ T cell infiltration. Moreover, immunonutrition induces an increase in epithelial cell functioning as APCs in patients not treated with neoadjuvant therapy. Finally, immunonutrition increased the postoperative blood circulating lymphocyte count at postoperative day 1. However, no differences in terms of disease-free survival or overall survival between patients who received immunonutrition and control group were observed. Therefore, immune modulating nutrition seems to activate the main immune surveillance mechanisms in healthy mucosa of patients having esophagectomy for cancer but the short duration of the supplementation prevents any conclusion on long-term effects. Thus, these results suggest a possible beneficial impact of immunonutrition on adenocarcinoma local recurrence after esophagectomy and encourage the design of adequately powered clinical trials.

Declaration of conflicting interests

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Supplementary material

Supplementary material is available for this article online

References

1. Jemal A, Murray T, Samuels A, et al. Cancer statistics, 2003. *CA: Cancer J Clin* 2003; 53(1): 5–26.
2. Gebski V, Burmeister B, Smithers BM, et al. Survival benefits from neoadjuvant chemoradiotherapy or chemotherapy in oesophageal carcinoma: a meta-analysis. *Lancet Oncol* 2007; 8(3): 226–234.
3. Greer SE, Goodney PP, Sutton JE, et al. Neoadjuvant chemoradiotherapy for esophageal carcinoma: a meta-analysis. *Surgery* 2005; 137(2): 172–177.
4. Zacherl J, Sendler A, Stein HJ, et al. Current status of neoadjuvant therapy for adenocarcinoma of the distal esophagus. *World J Surg* 2003; 27(9): 1067–1074.

5. Brücher BL, Stein HJ, Zimmermann F, et al. Responders benefit from neoadjuvant radiochemotherapy in esophageal squamous cell carcinoma: results of a prospective phase-II trial. *Eur J Surg Oncol* 2004; 30(9): 963–971.
6. Ruol A, Castoro C, Portale G, et al. Trends in management and prognosis for esophageal cancer surgery: twenty-five years of experience at a single institution. *Arch Surg* 2009; 144(3): 247–254.
7. De Vita F, Di Martino N, Orditura M, et al. Preoperative chemoradiotherapy for squamous cell carcinoma and adenocarcinoma of the esophagus: a phase II study. *Chest* 2002; 122: 1302–1308.
8. Enzinger PC and Mayer RJ. Esophageal cancer. *N Engl J Med* 2003; 349(23): 2241–2252.
9. Wu PC and Posner MC. The role of surgery in the management of oesophageal cancer. *Lancet Oncol* 2003; 4: 481–488.
10. Jamieson GG, Mathew G, Ludemann R, et al. Postoperative mortality following oesophagectomy and problems in reporting its rate. *Br J Surg* 2004; 91: 943–947.
11. Viklund P, Lindblad M, Lu M, et al. Risk factors for complications after esophageal cancer resection: a prospective population-based study in Sweden. *Ann Surg* 2006; 243(2): 204–211.
12. Braga M, Gianotti L, Vignali A, et al. Preoperative oral arginine and n-3 fatty acid supplementation improves the immunometabolic host response and outcome after colorectal resection for cancer. *Surgery* 2002; 132(5): 805–814.
13. Horie H, Okada M, Kojima M, et al. Favorable effects of preoperative enteral immunonutrition on a surgical site infection in patients with colorectal cancer without malnutrition. *Surg Today* 2006; 36(12): 1063–1068.
14. Vasson MP, Talvas J, Perche O, et al. Immunonutrition improves functional capacities in head and neck and esophageal cancer patients undergoing radiochemotherapy: a randomized clinical trial. *Clin Nutr* 2014; 33(2): 204–210.
15. Mabvuure NT, Roman A and Khan OA. Enteral immunonutrition versus standard enteral nutrition for patients undergoing oesophagogastric resection for cancer. *Int J Surg* 2013; 11(2): 122–127.
16. Kubota K, Kuroda J, Yoshida M, et al. Preoperative oral supplementation support in patients with esophageal cancer. *J Nutr Health Aging* 2014; 18(4): 437–440.
17. Galon J, Costes A, Sanchez-Cabo F, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science* 2006; 313(5795): 1960–1964.
18. Pagès F, Berger A, Camus M, et al. Effector memory T cells, early metastasis, and survival in colorectal cancer. *N Engl J Med* 2005; 353(25): 2654–2666.
19. Caglayan K, Oner I, Gunerhan Y, et al. The impact of preoperative immunonutrition and other nutrition models on tumor infiltrative lymphocytes in colorectal cancer patients. *Am J Surg* 2012; 204(4): 416–421.
20. Prieto I, Montemuiño S, Luna J, et al. The role of immunonutritional support in cancer treatment: current evidence. *Clin Nutr*. Epub ahead of print 24 November 2016. DOI: 10.1016/j.clnu.2016.11.015.
21. Scarpa M, Scarpa M, Castagliuolo I, et al. CD80 down-regulation is associated to aberrant DNA methylation in non-inflammatory colon carcinogenesis. *BMC Cancer* 2016; 16: 388.
22. Scarpa M, Scarpa M, Castagliuolo I, et al. Aberrant gene methylation in non-neoplastic mucosa as a predictive marker of ulcerative colitis-associated CRC. *Oncotarget* 2016; 7: 10322–10331.
23. Scarpa M, Pinto E, Saraceni E, et al. Randomized clinical trial of psychological support and sleep adjuvant measures for postoperative sleep disturbance in patients undergoing oesophagectomy. *Brit J Surg* 2017; 104: 1307–1314.
24. Rugge M, Correa P, Dixon MF, et al. Gastric dysplasia: the Padova international classification. *Am J Surg Pathol* 2000; 24(2): 167–176.
25. Bosman FT, Carneiro F, Hruban RH, et al. *world health organization classification of tumours of the digestive system*. Geneva, Switzerland: World Health Organization, 2010.
26. R Core Team. *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing, 2016.
27. Markar SR, Noordman BJ, Mackenzie H, et al. Multimodality treatment for esophageal adenocarcinoma: multi-center propensity-score matched study. *Ann Oncol* 2017; 28: 519–527.
28. Lou F, Sima CS, Adusumilli PS, et al. Esophageal cancer recurrence patterns and implications for surveillance. *J Thorac Oncol* 2013; 8(12): 1558–1562.
29. Scarpa M, Brun P, Scarpa M, et al. CD80-CD28 signaling controls the progression of inflammatory colorectal carcinogenesis. *Oncotarget* 2015; 6(24): 20058–20069.
30. Sultan J, Griffin SM, Di Franco F, et al. Randomized clinical trial of omega-3 fatty acid-supplemented enteral nutrition versus standard enteral nutrition in patients undergoing oesophagogastric cancer surgery. *Br J Surg* 2012; 99(3): 346–355.
31. Sato E, Olson SH, Ahn J, et al. Intraepithelial CD8+ tumor-infiltrating lymphocytes and a high CD8+/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer. *P Natl Acad Sci USA* 2005; 102(51): 18538–18543.
32. Talvas J, Garrait G, Goncalves-Mendes N, et al. Immunonutrition stimulates immune functions and antioxidant defense capacities of leukocytes in radiochemotherapy-treated head & neck and esophageal cancer patients: a double-blind randomized clinical trial. *Clin Nutr* 2015; 34(5): 810–817.
33. Fukuda T, Seto Y, Yamada K, et al. Can immune-enhancing nutrients reduce postoperative complications in patients undergoing esophageal surgery? *Dis Esophagus* 2008; 21(8): 708–711.