Acute changes in serum inflammatory signatures after consumption of ready-to-drink immuno-nutrition: a case report.

Modifications aiguës des signatures inflammatoires sériques après consommation d'immunonutrition prête à boire: à propos d'un cas clinique.

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Abstract

Background Immuno-nutrition administers specific nutrients to improve host defences, in particular supporting the immune system. Given the high invasiveness of some spine operations, strengthening the immune response could effectively balance the surgical stress and boost resilience. However, it is not clear what are the early molecular signatures after the consumption of immuno-modulatory nutrients.

Materials and methods In the context of nutritional prehabilitation, we present the case of a lady with scoliosis scheduled for surgery who was administered immuno-nutrition preoperatively and tested for acute changes in 200 biomolecules after two and four hours.

Results After four hours, we mainly observed 30-fold increase in IL-10, 10-fold increase in IL-18 inhibitory protein, 6-fold increase in IL-1 inhibitory receptor, 12-fold decrease in IL-2 receptor, and 51-fold decrease in IL-23. Gene ontology annotations showed the involvement of genes regulating cytokine activity, response to external organisms, humoral and adaptive response, and lymphocyte immunity.

Conclusion Overall, the trends over the first four hours showed a positive shift of the cytokine balance in favour of anti-inflammatory mediators while keeping active the cell-mediated immunity. The use of immuno-nutrition for prehabilitation patients could prove useful to patients but benefits from chronic consumption and surgical outcomes remain to be determined.

Keywords Orthopedic surgery; Immunomodulatory diet; L-arginine; Omega-3 fatty acid; Enhanced recovery after surgery.

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Résumé

Objectif L'immunonutrition administre des nutriments spécifiques pour améliorer le système immunitaire de l'hôte. Compte tenu du caractère hautement invasif de certaines opérations de la colonne vertébrale, le renforcement de la réponse immunitaire pourrait efficacement équilibrer le stress chirurgical et soutenir la résilience. Cependant, on ne sait pas exactement quelles sont les premières signatures moléculaires après la consommation de nutriments immunomodulateurs.

Matériels et méthodes Dans le contexte de la préhabilitation avant chirurgie majeure, nous présentons le cas d'une femme atteinte de scoliose programmée pour une intervention chirurgicale qui a consommé une immunonutrition préopératoire et a été testée pour détecter les changements de 200 biomolécules après deux et quatre heures de consommation.

Résultats Après quatre heures, nous avons observé principalement une augmentation de 30 fois de l'IL-10, une augmentation de 10 fois de la protéine inhibitrice de l'IL-18, une augmentation de 6 fois du récepteur inhibiteur de l'IL-1, une diminution de 12 fois du récepteur de l'IL-2 et une augmentation de 51 fois diminution de l'IL-23. Les annotations de l'ontologie des gènes suggèrent l'implication de gènes régulant l'activité des cytokines, la réponse aux organismes externes, la réponse humorale et adaptative et l'immunité lymphocytaire.

Conclusion Au cours dès les premières quatre heures, nous avons observé un déséquilibre de la réponse des cytokines en faveur des médiateurs anti-inflammatoires, maintenant ainsi active l'immunité à médiation cellulaire. L'immunonutrition chez les patients du rachis semble confirmer son rôle bénéfique sur le système immunitaire, mais les effets après une consommation chronique et l'impact sur les résultats chirurgicaux restent à déterminer.

Mots clés Chirurgie orthopédique; Régime alimentaire immunomodulateur; L-arginine; Acide gras oméga 3; Récupération améliorée après chirurgie.

1. Introduction

Adult spinal deformity is a spectrum of structural disorders of the musculoskeletal system of adult patients that mainly involves sagittal and coronal pathological shapes of the spine. In these conditions, there is an idiopathic or more often degenerative deviation from or interruption of the normal structure or function of the spine. Affected individuals often experience back pain, neurogenic claudication, radiculopathy, sciatica, and in severe curvatures gastroesophageal reflux and diminished lung function. Patients can be seen to tilt the head and neck back to level the gaze, straightens the thoracic spine to reduce kyphosis, and with the pelvis retroverted and knees flexed to compensate for forward-leaning [1]. Impaired function, undesirable appearance, and low self-esteem are often reported [2]. The ideal surgical treatment corrects both the sagittal and coronal misalignments and curvatures with operations that can last hours and require litres of blood to be transfused. Recovery is long and complex, and in the first few days after surgery complications can arise in up to 30% of patients [3], with predischarge neurologic injury, ileus, surgical site drainage, and pulmonary embolism being the most reported [4]. Surgical site infections occur in up to 8.5% of cases [5], drastically increasing mortality [6].

The potential of a patient's preoperative nutritional status to influence the outcome after spine surgery [7,8] has prompted clinical researchers to investigate dietary and nutritional strategies to be applied before elective interventions [9,10]. In particular, it is of most interest the practice of strengthening the first-line immune response and the second-line resilience against the consequences of the immune system hyperactivation with food forms or specific nutrients. This practice is termed "immuno-nutrition". Interventions on the spinal column, especially open surgeries, injure soft tissues and activate local inflammatory and neuroendocrine processes that pour into the bloodstream signalling peptides and proteins [11], causing a wide range of biological alterations that particularly affect the wound healing process [12]. Primarily, it is the content of ω-3 fatty acids, L-arginine, and nucleotides that gives the connotation of "immuno-nutrition" to a food product. The ω-3 fatty acids are known to down-regulate eicosanoid production, L-arginine to fuel the metabolic pathways of nitric oxide and positively modulate growth hormone, and to enhance the function of T-cells as do nucleotides [13]. However, there are other factors with acknowledged immunomodulatory effects: riboflavin (B2), pyridine compounds (B6), retinol (A), cholecalciferol (D3), α-tocopherol (E), trace elements such as zinc or selenium, and the conditionally essential amino acid glutamine [14]. While the studies that have observed the clinical effects are numerous, there is a lack of exploratory investigations aimed at uncovering the molecular mechanisms involved.

Here we report a case of a lady with scoliosis who was given immuno-nutrition before corrective surgery and whose serum inflammatory signatures were monitored in the four hours following consumption. The administration of immuno-nutrition was part of an ongoing trial design in which subjects undergoing major spine surgery had to be randomized to follow a pre-operative supplementation protocol with immuno-nutrition. The trial received approval on June 9th, 2021, from the San Raffaele Ethics Committee of Milan to be hosted and conducted at our IRCCS Orthopedic Institute Galeazzi. The study was registered on Clinicaltrials.gov (NCT05372289). Informed consent was obtained from the patient to be involved in the study. This publication adheres to the CAse REport (CARE) guidelines (care-statement.org).

2. Case presentation

The case is a lady who was referred to our hospital for elective spine surgery in May 2023. At the age of 50, she started to notice a sideways curvature tending to misalignment on the sagittal plane. The onset of lower back pain prompted her to plan to do more exercise. Over the years, the lack of benefit from increased structured physical activity and the worsening of painful symptoms pushed the patient to consult an orthopaedic surgeon who prescribed non-steroid anti-inflammatory drugs (NSAIDs) and corset wearing to improve pain and disability. Gradually, vertebral insufficiency aggravated, pain increased, and functional independence decreased. The lady therefore received the indication for surgical treatment at the age of 63.

At the pre-admission visit, the lady weighed 76.2 kg and the height on a wall-mounted stadium meter was 167.5 cm. She reported abstention from alcoholic beverages and smoking and denied any drug or food allergies. At that time, the lady was independent at home but needed help for outings, which were not frequent. It was the constant back pain rated 7 out of 10 that prevented her from carrying out normal daily activities. The lady had a primary lumbar curve and a visible hump due to hyperkyphosis but no spondylolisthesis. On physical examination, there was nothing to report regarding the abdomen or cardiac, vascular, and nervous systems. She had pulmonary sarcoidosis, ectopic pancreas and was pharmacologically treated for hypertension, hypercholesterolemia, and hypothyroidism. She reported to have been trying to eat healthy for a few weeks in preparation for the surgery. In particular, she reported consuming at least two servings of fruit and two of vegetables a day, basing meals on cereals from wholegrains, varying protein sources among seafood, legumes, and dairy products, and eating less meat or meat products. The Medi-Lite tool [15] scored 14, showing good adherence to the Mediterranean diet (0 = low adherence; 18 = high adherence). Although she reported having been following a healthy diet in the last weeks, the mini nutritional assessment-short form (MNA-SF) used for screening malnutrition [16]

indicated a risk presence (score of 10 out of 14), which mainly derived from the body mass index of 27.2 kg/m^2 and a reported recent weight loss of 2 kg. The bioimpedance analysis (BIA 101 BIVA® PRO, Akern s.r.l., Pisa, Italy) showed the following weight-related parameters: phase angle of 5, fat-free mass of 53.3 kg (73.8%), fat mass of 18.9 kg (26.2%), Hydragram® (ratio between total body water and fat-free mass) of 74.3%, and Nutrigram® of 739.8 (mg 24h/htm). The muscle quality index scored 0.3 and was derived from 8.5 kg of hand-grip strength measured on the non-dominant hand. Routine pre-operative clinical chemistry is displayed in **Table S1**. The patient was recruited in the randomized interventional trial on immuno-nutrition and allocated to the intervention group. She was given two cartons of 237 mL and instructed to drink in small sips but empty them within two hours. Three tubes of blood were collected two hours apart each. At the end of pre-operative checks, the lady has continued to follow the supplementation program for thirty days before surgery as required by the study design.

3. Materials and Methods

3.1. Oral nutritional supplement

The micro-ingredients of the oral nutritional supplement and their functions [13,14,17-25] are reported in **Table 1**. The macro-ingredients consisted of water, sucrose, milk proteins, maltodextrins, L-arginine, partially hydrolysed guar gum, fish oil, minerals, medium chain triglycerides, corn oil, acidifier, emulsifiers, yeast extract rich in nucleotides, choline bitartrate, flavourings, vitamins, stabilizers, antioxidant, and colouring. The supplement provided 341 kcal per carton of 237 mL, containing 76 g of water, 18.9 g of carbohydrates, 7.6 g of proteins, 3.9 g of fats, 1.4 g of fibres, and 0.38 g of salt. The supplement was classified by the manufacturing company (Nestlé Health Science Deutschland, Lyoner Strasse, Frankfurt, Germany) as a high-protein high-calorie nutritionally complete supplement that could help to strengthen the immune system in preparation for major surgery.

[Table 1]

3.2. Cytokine antibody array analysis

A blood specimen was collected from the lady in fasting condition and then after two and four hours from baseline. We used serum tubes with gel (BD Vacutainer® SST™ II Advance, REF 367955 of Belliver Industrial Estate, Roborough, Plymouth, United Kingdom), which were centrifugated at 2000 g for 15 min. at room temperature and then stored at -80°C till analyses. The multiplexed sandwich ELISA-based quantitative array platform (Quantibody® Human Cytokine Antibody Array 4000 of RayBiotech, Norcross, GA, United States of America) was used to measure the concentration of serum inflammatory factors. A 2-

fold sample dilution was used for the array according to the manufacturer's instructions (raybiotech.com/human-cytokine-array-q4000-qah-caa-4000), with the final output covering the amount of 200 human cytokines of the order of picograms per millilitres. It was projected to divide the factors into two groups, namely those who increased and those who decreased compared to baseline. The percent change between baseline and four hours would have been used to divide the proteins in each of the two groups into quartiles. The evaluation of functional annotations was intended to be performed only on the target factors ranking in the quartile revealing the largest deltas (below Q1 for decreasing factors and beyond Q3 for increasing factors). We used the on-line tool Gorilla (Gene Ontology enRIchment anaLysis and visuaLizAtion) to identify the enriched GO (Gene Ontology) terms in the corresponding genes compared to the background list of all the biomarkers.

4. Quantitative findings and inflammatory signatures

We excluded 14 factors (EpCAM, Fcg RIIBC, Follistatin, IL-17B, IL-2 Ra, SDF-1b, gp130, Siglec-5, IL-1 R4, TGFb2, Tie-2, TREM-1, VEGF-C, IL-11) from the analysis for detection errors. The most significant changes of the selected mediators are showed in **Table 2**. The complete list of the 4-hour change in all 200 biomolecules is shared immediately and indefinitely as **Supplementary Data** to the publication.

[Table 2]

The inflammatory signature associated with a GO term was built with 184 genes after removal of duplicate. In **Figure S1** are highlighted the biological processes and the molecular functions significantly represented in the pooled GO terms. In particular, annotations showed the significant involvement of genes regulating the molecular functions of cytokine activity and relative receptor bindings.

5. Discussion

There is a long history of research on the role of immuno-nutrients to enhance recovery after surgery. Agreed targets are mucosal barrier function (i.e. combined effects of extracellular and cellular processes with permeability primarily determined by tight junctions), cellular immune function (i.e. innate immune response comprising the multilayered mechanisms on which cells rely to spatially reorganise and synergistically cooperate against external organisms), and local and systemic inflammation [26]. The potential clinical benefit of using specific formula enriched with immuno-modulating substrates is mentioned in the ESPEN (European Society for Clinical Nutrition and Metabolism) guidelines for nutritional support in

major cancer surgery [27], upper gastrointestinal resection [28], and acute pancreatitis [29], with examined endpoints mostly being infectious rates, morbidity, and days of hospitalization. Immuno-nutrients are also discussed in the ERAS (Enhanced Recovery After Surgery) guidelines for perioperative care in lower extremity vascular bypasses [30], open aortic vascular surgery [31], gynaecologic/oncology [32], gastrectomy [33], cytoreductive surgery [34], and elective colorectal surgery [35]. Despite the acknowledgement of biological targets and the conduct of numerous clinical studies, there remains uncertainty on the mechanisms of action leading to the biological and clinical effects, resulting in a moderate level of evidence across different surgical specialties and a weak positive strength of recommendations.

In our case report, we focused on uncovering the molecular signatures early after the consumption of immuno-modulatory nutrients by a lady undergoing invasive surgery for spinal deformity. She was overweight, at risk of malnutrition, with a body composition tending towards an overhydration as informed by the Hydragram® (normal hydration between 72.8% and 74.3%), with a poor muscle quality as informed by the low index of 0.3 (normal value is greater than or equal to 1.5), and with a poor cell membrane integrity and body cell mass given the low standardized phase angle of -1.2 (normal value is greater than or equal to 0). Although the recent weight loss was reportedly voluntary, the autonomy with which the diet was set could not exclude an insufficient intake of some nutrients. The malnutritional status and the expected major surgery made the lady the ideal candidate to undergo the supplementation protocol.

Overall, we observed a riot of biological modifications that, although intricate to disentangle, it congruently aimed at reorganising cellular transport and cytokine receptor expression for activating cellular immune response. The cytokines at the two extremes of the range of 4-hour change in **Table 2** were IL-10 and IL-23. IL-10 augmented of +2848% from baseline, being the key anti-inflammatory cytokine inhibiting pro-inflammatory responses of both innate and adaptive immune cells. Contrarywise, IL-23 is a key cytokine for promoting inflammatory responses in a variety of target tissues and decreased of -98% from baseline. In brief, we also observed a 10-fold increase in the inhibitory protein of IL-18, a 6-fold increase in the inhibitory receptor of IL-1, and an 12-fold decrease in the receptor of IL-2. These modifications can be recognised as a preventive modulation of an early response of type 1 helper cells (Th1) to avert immune dysregulations. Th1 cells are generally considered pro-inflammatory immune cells involved in the fight against tumour cells and tissue injury, producing interferon-γ (INF-γ), IL-2, and tumour necrosis factor-α (TNF-α) to activate macrophages and other cells. The differentiation of helper T-cells into a specific type relies on circulating Th-cell-activating cytokines, such as IL-12 that promotes the differentiation into Th1.

Interestingly, we observed +785% of IL-12p70 and +463% of IL-12p40 that seem to concomitantly stimulate the activation of Th1 and natural killer (NK) cells. On the other hand, the Dickkopf-related protein 1 (DKK-1) augmented of +696%, which is known to inhibit the Wnt signalling pathway on which the activation of T cell transcription factor 1 (TCF1) relies on to control T-cell fate [36]. Besides the rise in IL-10, also the 616% increase in IL-4, which acts synergistically with IL-10 in suppressing inflammation, was indicative of a concomitant surge in polarized type 2 helper cells (Th2) cells. These cells are generally recognised as anti-inflammatory and involved in wound healing and immuno-suppression, secreting IL-13, IL-5, IL-4, and transforming growth factor actor-β (TGF-β) to activate the antibody-mediated immune response (i.e. B-cell recruitment). The thymic stromal lymphopoietin (TSLP) that is known to amplify the Th2 cytokine production, increased of 501%. IL-13, which is known to down-regulate IL-1 and TNF-α, raised of 7-fold but its two receptors R1 (low affinity) and R2 (high affinity) both decreased. Another T helper cell subset is the type 17 helper cells (Th17) that produces the cytokines IL-17A and IL-17F, which are known to confer protection against pathogens and keep mucosal immunity [37]. Both proteins rose of about 6-fold. Based on the GO terms analysis (**Figure S1**), immuno-nutrition showed to significantly regulate the biological processes linked to cytokine-mediated signalling pathway, defence response to bacteria and other organisms, positive regulation of release of sequestered calcium ion into cytosol, positive regulation of humoral immune response, positive regulation of adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains, and positive regulation of INF-γ production and lymphocyte-mediated immunity. For what concerns this latter aspect, it was confirmatory to note the increase in the chemokines monocyte chemoattractant protein (MCP)-1, -2, -3, and -4 as reported in **Table 2** and **Supplementary Data**, which play a major role in selectively recruiting various leukocytic cells that perpetuate the cellular response. Similarly, we observed a 5-fold rise in TNF-α, which is known to increase vascular permeability. Migration of immune cells from the bloodstream across the vascular endothelium is required for inflammatory response and immunological surveillance. The concomitant increase of both granulocyte macrophage- (GM-CSF) and macrophage-colony stimulating factors (M-CSF) was indicative of an ongoing polarization of macrophages, in our case tending to favour pro-inflammatory macrophages as evidenced by the shift of the ratio between GM-CSF/M-CSF from 0.9 to 2.83. However, these cells are a dynamic cell population and are known to reversibly undergo redifferentiation, with concomitant presence possibly being indicative of gene competition or even opposite responses [38]. Adult patients undergoing spinal deformity surgery normally face a postoperative increase in metabolic demands because of the trauma-derived inflammation. Balance of the biphasic post-operative pro- and anti-inflammatory responses

is necessary for healing, with pro-inflammatory cytokines having a critical role in orchestrating leukocyte infiltration, overall host response to injury, and normal immune response [39]. If inflammation is sustained after surgery, it may exert counterproductive effects and lead to depletion of the nutritional status, chronic post-surgical pain, and compromised recovery [40,41]. For instance, one of the roles NK cells is the control of aberrant chronic inflammation and unchecked cell destruction [42]. We observed an increase in IL-2, IL-4, and IL-7, IL-15, and IL-21, all of which are known to promote NK cell survival, proliferation, and functions [43]. The supplementation protocol also elicited a T-cell-mediated anti-tumour immune response, as proved by the acute reduction of -19% of angiogenin, which is known to promotes tumour growth [44].

The oral nutritional supplement delivered more than one immuno-modulatory element. For instance, the two cartons (474 mL) provided the following fractions of daily reference intakes: 73% of iron, 79% of zinc, 91% of copper, 45% of selenium, 101% of retinol, 29% of cholecalciferol, 182% of α-tocopherol, 150% of L-ascorbic acid, 74% of riboflavin, 63% of pyridine compounds, 40% of folic acid, 95% of cobalamin, and 120% of biotine. Therefore, we are not confident in ascribing a specific effect to a single component. Intracellular iron was shown in vitro to promote in T cells the expression of pro-inflammatory cytokines, such as GM-CSF and IL-2 [45], which we saw to increase by +984% from baseline. However, it is reasonable to exclude that taking iron orally could lead to a sudden change in its concentration inside cells. Lipid-soluble vitamins are well-absorbed from the intestine, but it is not known whether they can lead to perceptible effects in 4 hours. Cholecalciferol modulates in vitro dendritic cells differentiation and maturation, monocyte production of cytokines, B cell proliferation and differentiation, T cell maturation and proliferation, and Th1 to Th2 shift, resulting in decreased production of pro-inflammatory cytokines, such as IL-1, IL-6, IL-8, IL-12, IL-17, IL-21, and TNFα, and increased production of anti-inflammatory cytokines such as IL-10 [46,47]. The genes responsible for lymphocyte proliferation and survival are activated by α-tocopherol, modulating Th1 response [48], and the retinoic acid exerts several effects on the immunobiology of both myeloid and lymphoid cells [49]. Likewise, virtually each vitamin of the B group has an action on the immune system. Riboflavin was reported to influence the production of different inflammatory mediators, such as TNFα, IL-1β, MCP-1, INF-γ, IL-10 [23], and observations involving subjects with deficiencies of pyridine compounds [50], folic acid [51], biotin [19], and cobalamin [52] showed dysregulations in the immune system and inflammatory response. Other than micro-nutrients, the supplement provided calories that triggered an anabolic response as observed by the rise of 5-fold in insulin, the decrease in resistin of -65%, and reduction of -26% in agouti-related protein from baseline fasting condition. These calories could have corrupted the direct relationship between immuno-nutrients

intake and immune system modulation given the well-known role of energy accumulation and caloric restriction on inflammation [53,54]. Contrarywise, the energy contribution deriving from the nutritional supplement would have been beneficial in terms of caloric compensation in the event of insufficient intake. Definitely, administration of immuno-nutrients is advised to be maintained in the first days after surgery, when patients are known to lack appetite and eat scarcely [55].

To the authors' knowledge, this is the first time that the acute response of inflammatory factors, growth factors, chemokines, receptors, and cytokines to immuno-nutrition has been monitored in a patient with spinal deformity scheduled for major orthopaedic surgery (**Figure 1**). This report has limitations. First, the observed changes in inflammatory signatures cannot be generalized beyond the context of this case report, thus not posing any certain explanation to biological mechanisms of immuno-nutrients. Nevertheless, it is reasonable to think that the observed immune cascade is similarly elicited in other patients after consumption of the same oral nutritional supplement. Second, the investigated cytokines could be only a portion of biomarkers involved in inflammation and immune function, possibly overlooking other key factors involved in the observed changes (i.e. neopterin, lymphocyte phenotypes) [56]. Further aspects beyond clinical status, surgical stress, or dietary lifestyle are known to influence inflammation. For instance, smoking and chronic steroids negatively influence the healing process after spine surgery [57]. Since this report includes data from one patient with no control, it was not possible to evaluate the role of these aspects. Third, our findings are to be considered very short-term observations and what will happen after prolonged supplementation or what the impact will be on surgical outcomes cannot be forecasted. Fourth, it would have been useful to explore the presence of baseline low-grade inflammation or pre-existing micronutrient deficiencies before the administration of immuno-nutrients in order to fully understand the effects of any repletion treatment. To fully weigh the value of immuno-nutrition in prehabilitation, these aspects will need to be investigated. Fourth, prehabilitation ought to be multimodal and multidisciplinary. We cannot exclude that better outcomes may result from concomitant exercise therapy given the experienced ineffectiveness of single-modality interventions [58].

[Figure 1]

6. Conclusion

If cost-effective and with low safety risk profile, a nutritionally complete oral supplement that positively modulates the immune system can definitely be worthy of prescription compared to other more intensive

pre-optimisation options like diet therapy. It should be highlighted, however, that supplementation should not be offered to the patient as a long-term solution, which instead adherence to a healthy diet can be [59]. Nontargeted healthy eating lessons can be sufficient in case health professionals are conveying general concepts or in the event of group education sessions before surgery [15]. Nonetheless, a personalized approach may be more effective to deal with inter- and intra-variability [60]. Beyond the value from a clinical, health economic, patient-centred, and decision-making perspective [61], we support the importance of continuing to investigate the biological relevance coming from nutritional prehabilitation strategies. In particular, preoperative immuno-nutrition is a promising approach to rapidly enhance the cell-mediated immunity. Based on this case report, it is not possible to infer an existence of a cause-effect relationship between the administration of immuno-nutrients and the changes in serum inflammatory signatures. However, our exploratory report paves the way for future research, which will ought to study confounding factors or biases like biological circadian rhythms and examine how responses change from individual to individual and within the same subject under diverse circumstances. Tailored nutrition takes into account genetic variations and is procedure specific [62]. We must ensure a sensitive and specific allocation of immuno-nutrition to patients in order to provide the best care for the right patient at the right time.

Disclosure of interest

[redacted]

Funding

[redacted]

Authors' contributions

[redacted]

Data availability

The raw data of all biomarkers investigated in this case report is shared immediately and indefinitely as **Supplementary Data** to the publication.

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TABLES

Table 1 – Contents of the principal immuno-modulatory elements of the oral nutritional supplement along with the main immunological roles.

Ingredients	1 carton (237 mL)	Main immunological roles
ω-3 fatty acids	1422 mg	Modulation (down-regulation) of eicosanoid production.
L-arginine	4.3 g	Metabolic pathway of nitric oxide and growth hormone.
Nucleotides	0.43 g	Functioning of immune cells, in particular T-cells.
Iron	4 mg	Cell differentiation and growth; balance of Th1/Th2 ratio.
Zinc	5 mg	Activity of helper T-, NK-, and B-cells; Th1 response.
Copper	0.59 mg	Intracellular antioxidant balance; Th1 response.
Selenium	15.6 µg	Antibody production; response to external organisms.
Retinol	329 µg	Humoral antibody and cell-mediated response; gut integrity.
Cholecalciferol	2.2 µg	Cell proliferation; up-regulation of anti-inflammatory cytokines.
α-tocopherol	10 mg	Th1 response; NK-cells activity; reduction of prostaglandin E2.
L-ascorbic acid	71 mg	NK and lymphocyte proliferation, chemotaxis; redox integrity.
Riboflavin	0.59 mg	Phagocytosis and proliferation; anti-inflammatory property
Pyridine compounds	0.5 mg	Lymphocyte activity; Th1 immune response; antibody production.
Folic acid	66 µg	NK-cell activity, proliferation, and survival; cytokine production.
Cobalamin	1.9 µg	Activity of cytotoxic cells (NK and CD8+ T-cell lymphocytes.
Biotine	24 µg	Activity of T- and NK-cells; balance of Th1/Th17 ratio.

Notes: Th1 = type 1 helper cells; Th2 = type 2 helper cells; Th17 = type 17 helper cells; NK = natural killer cells.

Table 2 – Major quantitative variations in inflammatory factors, growth factors, chemokines, receptors, and cytokines after immuno-nutrition.

Factor/target	Gene	Factor/target	Biological function*	Baseline	2 hours	4 hours	4-h trend
acronym	*name*	*extended name*	*description*	*(pg/mL)*	*(pg/mL)*	*(pg/mL)*	*%*
IL-10	IL10	Interleukin-10	Regulatory cytokine that limits tissue disruption caused by inflammation. It targets macrophages and monocytes to inhibit the release of GM-CSF, IL-1α, IL-1 β, IL-6, IL-8, and TNFα.	12	487	363	+2848%
GM-CSF	CSF2	Granulocyte macrophage-colony stimulating factor	Cytokine that stimulates the growth and differentiation of haematopoietic precursor cells from various lineages, including granulocytes, macrophages, eosinophils and erythrocytes.	13	353	376	+2840%
Lymphotactin	XCL1	Lymphotactin	Chemotactic mediator for lymphocytes. In thymus, it regulates medullary accumulation of dendritic cells and contributes to regulatory T cell development, playing a role in self-tolerance.	632	8236	9193	+1355%
MIG	CXCL9	Monokine induced by interferon-γ	Cytokine that affects the growth, movement, or activation state of cells that participate in immune and inflammatory responses. It has a chemotactic role for activated T-cells.	1166	10733	15396	+1220%
IL-28A	IFNL2	Interleukin-28A	Tissue-specific cytokine with antiviral, antitumour, and immunomodulatory activities. It plays critical role in antiviral host defence and acts as a ligand for IL-10 RB and IFNL R1.	234	1471	2672	+1043%
MCP-3	CCL7	Monocyte chemoattractant protein-3	One of the most pluripotent chemokines that acts as a chemotactic factor for monocytes and eosinophils. It augments monocyte anti-tumour activity and plays a role in homeostasis.	125	1202	1424	+1038%
MSP	MST1	Macrophage stimulatory protein	It exerts dual effects on macrophages, inducing their spreading, migration, and phagocytosis but, on the other hand, inhibiting the production of inflammatory mediators, including prostaglandins.	1045	6619	11836	+1032%
IL-8	CXCL8	Interleukin-8	It is one of the major mediators of inflammatory response, being a chemotactic factor that attracts neutrophils, basophils, and T-cells to clear pathogens and protect the host from infection.	199	1477	2255	+1032%

Table 2 – *Cont.*

Factor/target	Gene	Factor/target	Biological function*	Baseline	2 hours	4 hours	4-h trend
acronym	*name*	*extended name*	*description*	*(pg/mL)*	*(pg/mL)*	*(pg/mL)*	*%*
MCP-2	CCL8	Monocyte chemoattractant protein-2	One of the key chemotactic factors that regulates migration and infiltration of monocytes, lymphocytes, basophils, and eosinophils. It plays important role in neoplasia and inflammatory host responses.	42	394	458	+996%
MIP-3α	CCL20	Macrophage inflammatory protein-3α	Induces a strong chemotactic response of immature dendritic cells, effector/memory T-cells, and B-cells to sites of inflammation. It appears to have a direct antiviral and antifungal activities.	20	171	215	+987%
IL-2	IL2	Interleukin-2	Cytokine produced by activated CD4+ helper T-cells that plays a pivotal role in the immune response and tolerance, such as the proliferation of B-cells and regulatory T-cells.	83	836	902	+984%
CD30	TNFRSF8	Lymphocyte activation antigen CD30	Antigen member of the TNF receptor superfamily that is stimulated by IL-4 and expressed by a subset of T-cells. It regulates cellular growth and transformation of activated lymphoblasts.	798	9654	8192	+927%
G-CSF	CSF3	Granulocyte-colony stimulating factor	Cytokine that acts as a blood growth factor. In hematopoiesis, it controls the production, differentiation, and function of granulocytes and monocytes-macrophages.	250	2310	2545	+916%
IL-18 BP	IL18BP	Interleukin-18 binding protein	High affinity isoform that binds to the pro-inflammatory cytokine IL-18 and inhibits its activity. It functions as an inhibitor of the early Th1 cytokine response by binding to IL-18 and reducing INFγ induction.	1107	9561	11117	+904%
LIGHT	TNFSF14	Tumour necrosis factor ligand superfamily member 14	It functions as a co-stimulatory factor for the activation of lymphoid cells. Its binding delivers signals to T-cells and leads to T-cell proliferation, IFNγ production, and apoptosis of various tumour cells.	925	4105	8871	+859%
M-CSF	CSF1	Macrophage-colony stimulating factor 1	Cytokine that plays essential role in survival, proliferation, and differentiation of macrophages and monocytes. It has a role in innate immunity and inflammation and in bone cell differentiation.	14	118	133	+833%

Table 2 – *Cont.*

Factor/target	**Gene**	**Factor/target**	**Biological function***	**Baseline**	**2 hours**	**4 hours**	**4-h trend**
acronym	*name*	*extended name*	*description*	*(pg/mL)*	*(pg/mL)*	*(pg/mL)*	*%*
IL-12p70	IL12A/IL12B	Interleukin-12 subunit α/β	Heterodimer produced mainly by macrophages and dendritic cells enhancing antigen presentation. It binds subunits of IL-12, activating Th1 cells, subsets of T-cells, NK, innate lymphoid cells.	3	27	28	+785%
TNFβ	LTA	Tumour necrosis factor ligand superfamily member 1	Cytokine with homology with TNFα. It is produced by lymphocytes and plays important roles in immune response to pathogens. It is cytotoxic for a wide range of tumour cells.	289	3875	2366	+719%
DKK-1	DKK1	Dickkopf-related protein 1	Secreted protein that acts as an inhibitor of the Wnt signalling pathway, directly regulating several immune cells. It regulates development, bone formation, cancer and anti-apoptotic activity.	92	558	731	+696%
CTACK	CCL27	Cutaneous T-cell-attracting chemokine	Chemotactic factor that attracts skin-associated memory T-lymphocytes and subtypes of dendritic cells and eosinophils. It plays a role in mediating this process to cutaneous sites.	713	6131	5662	+694%
TECK	CCL25	Thymus-expressed chemokine	Protein constitutively expressed in the thymus and small intestine. It shows chemotactic activity on thymocytes, macrophages, dendritic cells, and is potentially involved in T-cell development.	794	6582	6255	+688%
IL-13	IL13	Interleukin-13	Cytokine with similar function of IL-4. It is secreted by activated T-cells and plays roles in allergic inflammation and immune response to infection. It down-regulates IL-1 and TNF-α.	25	172	182	+634%
IL-4	IL4	Interleukin-4	Cytokine primarily secreted by mast cells, T-cells, eosinophils, and basophils. It regulates antibody production and inflammation. It acts synergistically with IL-10 in suppressing inflammation.	515	3068	3685	+616%
IL-1β	IL1B	Interleukin-1 β	Potent pro-inflammatory cytokine that induces prostaglandin synthesis, neutrophil, T-cell, B-cell activation, cytokine production. Response mediated by IL-1 R1 and inhibited by IL-1 R2	269	1656	1791	+565%

Table 2 – *Cont.*

Factor/target	**Gene**	**Factor/target**	**Biological function***	**Baseline**	**2 hours**	**4 hours**	**4-h trend**
acronym	*name*	*extended name*	*description*	*(pg/mL)*	*(pg/mL)*	*(pg/mL)*	*%*
IL-2 Rγ	IL2RG	Interleukin-2 receptor subunit γ	Common subunit for the receptors for a variety of interleukins, such as IL-2, IL-4, IL-7, IL-9, IL-21. It is involved in neutrophil phagocytosis, T-cell differentiation, activation, survival.	245	1812	1621	+561%
I-TAC	CXCL11	Interferon-inducible T-cell α chemoattractant	Chemotactic for interleukin-activated T-cells, neutrophils, or monocytes during IFN-dominated immune response. Regulated by INF and may play a role in tissue-specific immune responses.	927	3252	6116	+558%
VEGF-D	VEGFD	Vascular endothelial growth factor-D	Secreted growth factor that activates VEGF receptors, promoting remodelling of adult vessels. It is active in angiogenesis and endothelial cell growth, stimulating proliferation and migration.	868	6326	5620	+547%
IL-1 R1	IL1R1	Interleukin-1 receptor type 1	It is a member of the Toll-like receptor superfamily. It is the receptor of IL-1 that mediates the IL-1-dependent activation of specific pathways involving NK-kappa-B, MAPK, and others.	381	2034	2380	+524%
IL-17F	IL17F	Interleukin-17F	Member of the IL-17 family that is associated with activated CD4 T-cells, monocytes, basophils, and mast cells. It is involved in innate/adaptive immune system, host defence, tissue integrity.	3605	24529	22335	+520%
TSLP	TSLP	Thymic stromal lymphopoietin	Epithelial cell-derived cytokine expressed that induces the release of T-cell-attracting chemokines and the maturation of CD11c(+) dendritic cells. It amplifies Th2 cytokine production.	405	3046	2434	+501%
IL-17A	IL17A	Interleukin-17A	Principal effector of Th17 cells, it is a cytokine participating in innate and adaptive immune system. It is involved in antimicrobial host defence and maintenance of tissue integrity.	22	81	133	+501%
IP-10	CXCL10	10 kDa interferon γ-induced protein	Pro-inflammatory cytokine produced by T-cells, monocytes, and endothelial cells. It is involved in chemotaxis, differentiation and activation of peripheral immune cells, growth and apoptosis.	1160	7381	6785	+485%

Table 2 – *Cont.*

Factor/target	Gene	Factor/target	Biological function*	Baseline	2 hours	4 hours	4-h trend
acronym	*name*	*extended name*	*description*	*(pg/mL)*	*(pg/mL)*	*(pg/mL)*	*%*
NRG1-b1	NRG1	Pro-neuregulin-1	Isoform of NRG1, which is a multifunctional and versatile protein playing fundamental roles during development, structural maintenance, and functional integrity of several tissues.	2092	13818	12184	+482%
SDF-1a	CXCL12	Stromal cell-derived factor 1	Chemoattractant expressed by several cell types, involving osteoblasts, fibroblasts, and endothelial cells. It is active on T-lymphocytes and monocytes, regulating migration and adhesion.	114	499	656	+476%
IL-12p40	IL12B	Interleukin-12 subunit β	Subunit of the IL-12 family of cytokine, which associates with the p35 to form IL-12 and with p19 to form IL-23. It is a growth factor for activated T and NK cells that enhance lytic activity and IFN-γ.	278	1530	1566	+463%
BDNF	BDNF	Brain-derived neurotrophic factor	Key protein that promotes the survival and differentiation of selected neuronal populations of the peripheral and central nervous system. Expression increases in enriched environment.	79	793	428	+442%
TNFα	TNF	Tumour necrosis factor α	Inflammatory cytokine mainly secreted by macrophages that induces diverse signalling events, leading to apoptosis/necrosis. It is a potent pyrogen causing fever by stimulation of IL-1.	304	1247	1646	+441%
MICA	MICA	MHC class I polypeptide-related sequence A	A highly polymorphic complex that acts as a stress-induced self-antigen recognized by γ delta T-cells. Its binding triggers activation of NK cells against infected cells, causing cell lysis.	2866	16439	15178	+430%
AR	AREG	Amphiregulin	Ligand of the epidermal growth factor receptor that stimulates cell growth, survival, and migration of a broad range of target cells including fibroblasts. It modulates immunity.	635	3749	3359	+429%
GCP-2	CXCL6	Granulocyte chemotactic protein-2	Chemokine with chemotactic and pro-angiogenic properties similar to IL-8. It has a strong antibacterial activity against Gram-positive and Gram-negative bacteria, regulating neutrophil recruitment.	209	1177	1095	+423%

Table 2 – *Cont.*

Factor/target	Gene	Factor/target	Biological function*	Baseline	2 hours	4 hours	4-h trend
acronym	*name*	*extended name*	*description*	*(pg/mL)*	*(pg/mL)*	*(pg/mL)*	*%*
IL-1 R2	IL1RN	Interleukin-1 receptor antagonist protein	Anti-inflammatory antagonist of IL-1 family of pro-inflammatory cytokines, including IL-1α and IL-1β. It protects from immune dysregulation and systemic inflammation, mostly in the acute phase.	440	1856	2295	+422%
IL-13 R1	IL13RA1	Interleukin-13 receptor subunit α-1	Low affinity receptor that mediates the actions of IL-13. Similarly, it serves as an accessory protein to the cytokine receptor γ chain for IL-4, enabling cytokine binding/signalling.	1416	8180	123	-91%
IL-2 Rβ	IL2RB	Interleukin-2 receptor subunit β	Receptor for IL-2 that is involved in the mitogenic signals of IL-2 and in the stimulation of neutrophil phagocytosis by IL-15. Its role is similar to IL-2 Rγ, in charge of signalling.	6140	36770	514	-92%
PAI-1	SERPINE1	Plasminogen activator inhibitor-1	Primary inhibitor of plasminogen activator required for fibrinolysis down-regulation and controlled degradation of blood clots. Role in cell migration, spreading, adhesion.	4620	6592	335	-93%
VEGF R1	FLT1	Vascular endothelial growth factor receptor 1	Receptor for VEGF-A, PlGr, and VEGF-B. It plays a negative role in angiogenesis. It regulates cell survival and migration, macrophage function, chemotaxis, and cancer cell invasion.	71838	374554	1572	-98%
IL-23	IL23A	Interleukin-23 subunit A	Heterodimer composed subunits IL-23A and IL-12B. Association with IL12B forms the pro-inflammatory IL-23, stimulating the production of IL-17 in the subpopulation of CD4 helper (Th) cells called Th17.	36528	98267	719	-98%
PDGF-AB	PDGFA/PDGFB	Platelet-derived growth factor subunit A/B	Protein consisting of two distinct chains (A and B) that acts mainly on connective tissue cells. It is a potent activator for cells of mesenchymal origin stimulating chemotaxis, proliferation, and activation.	3448	7956	1	-100%

Notes: The functional information of proteins was retrieved from the freely accessible on-line resource uniprot.org (accessed between July 7-11, 2023).

FIGURES

Figure 1 – Summary slide of the case report aimed at uncovering the acute changes in serum inflammatory signatures after consumption of ready-to-drink immuno-nutrition.

SUPPLEMENTARY DATA

Table S1 – Routine laboratory results of the case.

Supplementary – Raw Data.

Figure S1 – The inflammatory signature associated with a GO term that emerged from the pooled 4-hour variations of selected proteins after the consumption of immuno-nutrition. **Notes:** The ontologies of the biological process and molecular function consist of a directed acyclic graph where the significantly enriched GO terms are highlighted according to the significance of the detected enrichment.

Supplementary – CARE checklist.

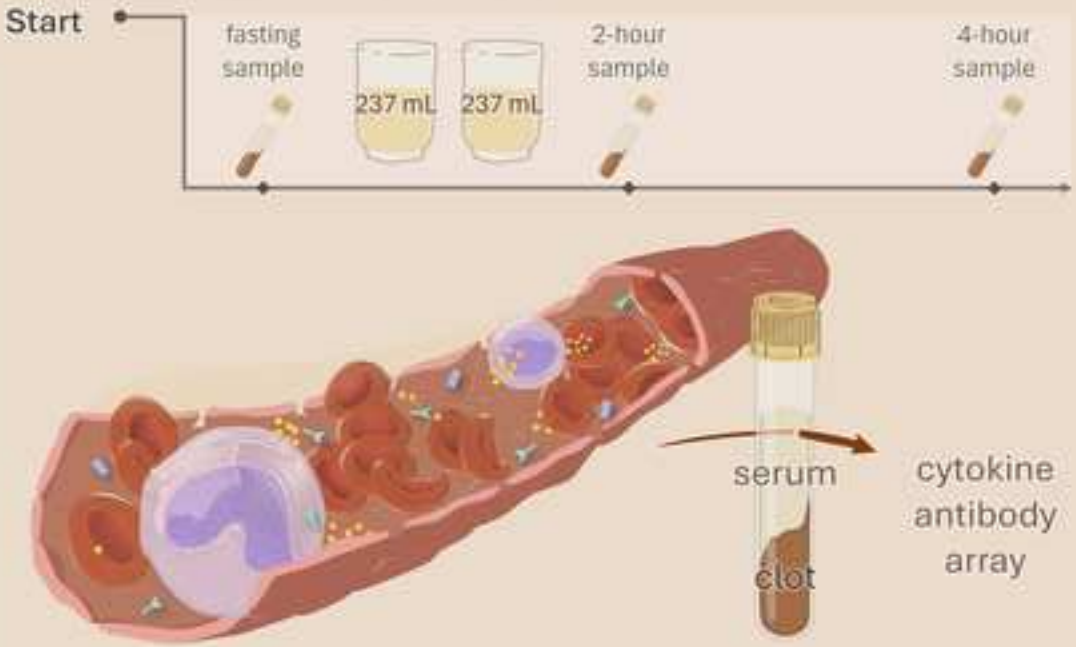
Figure 1

Acute changes in serum inflammatory signatures after consumption of ready-to-drink immuno-nutrition: a case report

Main ingredients of the drink

Sucrose, milk protein, maltodextrins, L-arginine, fish oil, minerals, medium-chain triglycerides, yeast extract rich in nucleotides, choline, and vitamins.

Experimental protocol



Critical signatures observed

30-fold increase in IL-10; 10-fold increase in the inhibitory protein of IL-18; 6-fold increase in the inhibitory receptor of IL-1; 12-fold decrease in the receptor of IL-2; 51-fold decrease in IL-23

Major functional annotations involved

- innate response
- cytokine activity
- response to external organism
- humoral response
- adaptive response
- lymphocyte immunity

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