Algemene gegevens / General Information

Programma / Programme	:	**COVID-19 Programma**
Subsidieronde / Subsidy round	:	**Bottom-up ronde COVID-19 aandachtsgebied 1**
Projecttitel / Project title	:	**MICRONUTRI-OMICS in patients with COVID-19 pneumonia**
Projecttaal / Project language	:	**Engels / English**
Geplande startdatum / Planned start date	:	**15-06-2020**
Geplande duur / Planned duration	:	**24 maanden / months**
Datum indienen / Date of application	:	**14-05-2020**
Projecttype / Project type	:	**Toegepast onderzoek**
Vervolg eerder ZonMw-project / Continuation previously funded project ZonMw	:	**Ja / Yes**
Eerder gehonoreerd projecttype / Project type of previously funded project	:	**Toegepast onderzoek**

Projectleden / Project members

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Department of Clinical Chemistry
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Dr. (10)(2e) **(Project commission member)**
Functie / Position: Intensivist | *Opleiding / Education:*
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Projectgegevens / Project information

Aandachtsgebieden / Focus

1.1 Thema's aandachtsgebied 1
- Risicoanalyse en prognostiek

1.3 Setting
- Ziekenhuiszorg

Samenvatting / Summary

More than 250,000 people have died, because there is no proven therapy for COVID-19 infection. Supplementation of micronutrients is one of the core pillars of treatment measures for COVID-19, because micronutrients play a key role in innate and adaptive immunity. Patients with a high risk of micronutrient deficiency have a worse prognosis. However, determination of micronutrient plasma concentrations is not available in daily practice. Covering basal daily required intakes of micronutrients is not sufficient in critically ill patients due to increased needs, especially in COVID-19. Timing, duration and dosing are crucial for optimal clinical benefit. Combined micronutrient supplementation has been associated with improved survival. There is a wide variation in protocols for micronutrient administration. Targeted detection-correction of multiple micronutrients could be a major step forward: rapid determination of the micronutrient status in individual patients and subsequent titrated supplementation. First, we will develop and validate a multiparameter vitamin assay with mass spectrometry that we will apply to biobank samples of ICU-patients with COVID-19, together with conventional trace elements profiling. Next this MICRONUTRI-OMICs approach will be applied for determination of vitamins in critically ill patients with COVID-19 pneumonia. The results will be applicable immediately to daily practice, because we will know whether the current practice of supplementation is sufficient to

normalize micronutrient plasma concentrations in our patients, or whether we should increase and/or prolong the supplementation. The results of this project will be the base for an RCT comparing targeted detection-correction of multiple micronutrients with standard of care. In this RCT we will apply AutoKinetics (a clinical decision support system) for micronutrient dosing. The results of our project will be relevant for every other new pandemic and also for the general ICU-population.

Trefwoorden / Keywords

Micronutrients, COVID-19, vitamins, trace-elements, critical care

Samenwerking / Collaboration

Samenwerking tussen onderzoek en praktijk / Cooperation between research and practice:

Ja / Yes

Organisaties

Amsterdam UMC - locatie VUmc
Medical Oncology
OncoProteomics Laboratory

Amsterdam UMC - locatie VUmc
Department of Clinical Chemistry

Amsterdam UMC - locatie VUmc
Intensive Care
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Inhoud / Content

Disciplines / Disciplines

- Geneeskunde, overig / Medicine, other

Financiële gegevens / Financial data

ZonMw budget

	Jaar / Year								
Kostenpost	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	**Totaal / Total**
Personeel	(10)(1c)								
Materieel									
Implementatie									
Apparatuur									
Overig									
Totaal / Total									

Co-financiering / Cofinancing

Naam co-financier / Name of cofinancier	Bedrag / Amount	Status

Bijzondere gegevens / Additional information

Vergunningen / Permits

	Verklaring nodig / Statement required?		Status verklaring / Statement status		
	Ja / Yes	Nee / No	Verkregen / Acquired	Aangevraagd / Applied	Nog niet aangevraagd / Not applied yet
METC	X				X
DEC		X			
WBO		X			

Onderschrijvingen / Assents

	Ja / Yes	Nee / No	N.v.t. / N.A.
Code biosecurity / Code Biosecurity		X	
Code openheid dierproeven / Code Transparency of Animal Testing		X	

Andere vergunningen / Other permits

AANVRAAGFORMULIER PROJECTIDEE – BOTTOM-UP RONDE

COVID 19 programma

Deadline voor indiening: 14 mei 2020 (14:00 u)

LEES ALSTUBLIEFT ALLE INSTRUCTIES IN BIJLAGE "TOELICHTING INDIENING PROJECTIDEE" VAN DE OPROEPTEKST ZORGVULDIG!

Wanneer u het formulier heeft ingevuld:

1. **Zet het formulier om naar een PDF file en controleer de details**
2. **Upload het complete formulier als een bijlage bij uw indiening in Projectnet (Let op: dit zijn twee verschillende links, gebruik maar 1 van de 2!)**

ProjectNet: Aandachtsgebied 1 (voorspellende diagnostiek en behandeling)
ProjectNet: Aandachtsgebied 2 (zorg en preventie)

BASISGEGEVENS (voorpagina)

NAAM VAN DE HOOFDAANVRAGER:

Dr. (10)(2e)

ORGANISATIE:

Amsterdam UMC, location VUmc

PROJECTTITEL:

MICRONUTRI-OMICS in patients with COVID-19 pneumonia

DATASTEWARD:

Wie is de datasteward die de open science en FAIR data planning in uw project ondersteunt? Zie de webinars op de ZonMw website om de datastewards te informeren en ondersteunen.

☒ Ik betrek een datasteward bij mijn project:
Naam: Dr. (10)(2e)
Instituut: Amsterdam UMC
E-mail: (10)(2e)@vumc.nl
Was aanwezig bij de webinar: ☐ Ja ☒ Nee

☐ Ik heb nog geen datasteward.

ONDERZOEKSVOORSTEL max 3 pagina's A4 (inclusief literatuurreferenties)	(voorpagina met basisgegevens niet meegerekend - font type Arial 10 pts)

1. PROBLEEMSTELLING EN DOELSTELLING(EN):

Currently, more than 250,000 people have lost their lives, because there is no proven therapy for COVID-19 infection. Supplementation of micronutrients is among the mainstay of general supportive treatments and one of the core pillars of comprehensive treatment measures for patients with COVID-19, because micronutrients play a key role in innate and adaptive immunity (table 1, [1-3]). Despite people of all ages can become infected, patients with a high risk of micronutrient deficiency (malnourished elderly people and patients with chronic diseases) have a worse prognosis and higher mortality rates [1]. The prevalence of malnutrition in elderly patients with COVID-19 was high in Wuhan [4]. However, the determination of micronutrient plasma concentrations is laborious, expensive and not available in daily practice. Furthermore, establishing exact micronutrient requirements in the critically ill has proven to be notoriously difficult. Covering the basal current daily required intakes of micronutrients is probably not sufficient in critically ill patients, who have increased needs due to illness induced decreased intake, increased losses, drug interactions with (single) micronutrients and hypermetabolism. This applies especially to patients with COVID-19, who frequently develop ARDS, a katabolic state, and acute kidney injury necessitating renal replacement therapy with inevitable loss of useful molecules. Moreover, these patients often have a prolonged length of stay on the Intensive Care Unit of 3 weeks or longer. The current evidence suggests that timing (as early as possible), (sufficient) duration, and dosing are key factors to ensure optimal clinical benefit for several micronutrients [5, 6]. In addition, it is important to emphasize that micronutrients do not act alone but as the part of a complex web. Providing a single element may shift the balance, or not achieve the maximum effect resulting from a combination. Combined micronutrient supplementation has been associated with a decrease in mortality, whereas provision of single micronutrients were associated with borderline statistical significance [6]. Finally, optimal plasma concentrations during the acute phase of disease remain presently uncertain. Nowadays, there is a wide variation in the presence and content of protocols for micronutrient administration. We hypothesize that *targeted detection-correction* of multiple micronutrients could be a major step forward: rapid determination of the micronutrient status in individual patients and subsequent titrated supplementation. This may be superior to empirical higher dose administration, since prolonged administration of unnecessarily large doses may be toxic, particularly for iron, copper, selenium, zinc, and the vitamins A and E (figure 1).

Epithelial barriers	Cellular immunity	Antibody production
Vitamin A	Vitamin A	Vitamin A
Vitamin C	Vitamin B_6	Vitamin B_6
Vitamin E	Vitamin B_{12}	Vitamin B_{12}
Zinc	Vitamin C	Vitamin D
	Vitamin D	Vitamin E
	Vitamin E	Folic acid
	Folic acid	Zinc
	Iron	Copper
	Zinc	Selenium
	Copper	
	Selenium	

Table 1 Summary of the sites of action of micronutrients on the immune system

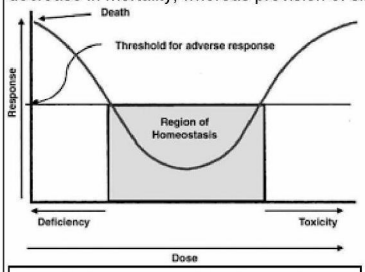


Figure 1: Dose-response relations for essential vitamin or mineral nutrients. Hayes Am J Clin Nutr 2008

Aim of this project:

To develop a multiparameter micronutrient assay with mass spectrometry for *targeted detection-correction* of multiple micronutrients in critically ill patients with COVID-19 pneumonia.

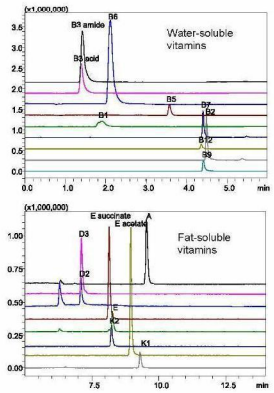
2. PLAN VAN AANPAK:

Sub-aim 1: (Month 1 – 6) Development of multi-parameter mass spectrometry assay for comprehensive determination of water-soluble and fat-soluble vitamins in patient plasma:

Water- soluble and fat-soluble vitamins in clinical samples are currently analysed separately using various methods, which is very time-consuming and laborious when a variety of vitamins needs to be measured. Therefore, we will develop and implement a multiparameter vitamin assay that can simultaneously analyse multiple water-soluble and fat-soluble vitamins in patient plasma. To this end, we will use an on-line Liquid Chromatography tandem Mass Spectrometry method using the proven, accurate quantitation capabilities of a Triple Quad™ mass spectrometer (SCIEX QTrap5500). This instrument has enhanced scan functions to enable simultaneous quantitation and confirmation at low level. Vitamins will be extracted using appropriate solvents as previously established. One assay will be developed for the following water-soluble vitamins:

Thiamin (B1), Riboflavin (B2), Nicotinic acid Nicotinamide (B3) Pantothenic acid (B5), Pyridoxine (B6), Biotin (B7), Folic acid (B9), Ascorbic acid (C) and Cyanocobalamin (B12) and one assay for the following fat-soluble vitamins: Retinol (A) β-carotene (proA) Ergocalciferol (D2), D3, α, β, γ-tocopherol (E), Phytonadione (K1), and Menaquinone -4, 7(K2). Predetermined Multiple Reaction Monitoring (MRM) transitions will be used to develop a multiparameter method for quantitative analysis of the vitamins. Three optimized MRM transitions were selected for each vitamin, one as quantifier ion and the others as confirmation ions. A gradient elution method will be set up for separation of both water-soluble vitamins and fat-soluble vitamins in one run, aiming to retain and separate the more polar analytes in the initial part of the gradient while the latter part of the gradient will elute and separate the fat-soluble vitamins using reversed phase conditions. Calibration curves will be made using vitamin standards and labelled standards spiked into biological matrix.

Total MRM chromatograms



Multi-parameter mass spectrometry assay for simultaneous detection and quantification of water- and fat-soluble vitamins in plasma

Sub-aim 2: (Month 7 –8) Validation of new multi-parameter vitamin assay

We will bench-mark limit of detection and quantification of the new multiparameter assay to the conventional methods and explore repeatability. The routine measurements will be performed by the department of Clinical Chemistry with routine methods under ISO15189 accreditation using High Performance Liquid Chromatography (HPLC), mass spectrometry (LC-MS/MS), colorimetry, competitive immune assays and inductively coupled plasma-mass-spectrometry (ICP-MS). The final multiparameter assay for water- and fat-soluble vitamins will have good linearity (R2>0.99) and a repeatability of less than 5% variation and is expected to outperform the conventional methods in both accuracy and speed.

Sub-aim 3 (month 8 -12): MICRONUTRI-OMICs of biobank samples of ICU-patients with COVID-19

In heparin and serum samples of 25 ICU-patients with COVID-19 collected on day 1, 3, 5, 7, 14 and 21 of their hospital stay, we will perform the new multi-vitamin assay and targeted analysis of trace elements. We will determine the micronutrient levels in a control group of 25 ICU-patients with non-COVID-pneumonia as well for comparison. We will investigate the association of vitamin and trace elements to nutritional status, actual supplementation, kidney function, Renal Replacement Therapy, liver function, fluid balance, severity of organ dysfunction and comorbidity.

Sub-aim 4: (Month 10 – 21) Prospective clinical study using MICRONUTRI-OMICs of COVID-19 patients receiving standard micronutrients supplementation protocol

Micronutrient profiling using the new multivitamin assay and targeted analysis of trace elements will be applied to new patient samples towards the end of year 1 and in year 2. We aim to include 50 patients of whom blood will be sampled at multiple time points. Clear deficiencies of the targeted micronutrients will lead to reevaluation and potentially adjustment of the doses used in our standard protocol. Currently, all our ICU-patients receive once a day Cernevit

Table 2: Cernevit

Vitamin A	3500 IU
Biotin	69 µg
Folic acid	414 µg
Vitamin B12	6 µg
Thiamine	3.51 mg
Riboflavin	4.14 mg
Vitamin B6	4.53 mg
Panthotenic acid	17.25 mg
Vitamin C	125 mg
Vitamin D	220 IU (D3)
Vitamin E	10.2 mg

Table 3: Supliven

Chlorium chloride hexahydrate	53.3 µg
Potassium jodide	166 µg
Cupper chloride dihydrate	1.0 mg
Manganese chloride tetra hydrate	0.198 mg
Sodium fluoride	2.1 mg
Sodium molybdate dihydrate	48.5 µg
Sodium selenite anhydrate	173 µg
Ferric chloride hexahydrate	5.4 mg
Zinc chloride	10.5 mg

(table 2) and Supliven (table 3) during the first 5 days of their ICU-stay, followed from the 6th day with Supradyn complex forte. We will investigate whether this supplementation is sufficient to normalize plasma concentrations.

3. HAALBAARHEID VAN HET PROJECT:

The time schedule of our project has been outlined in the former section. Since we have a lot of collected blood samples of COVID-19 patients stored in biobank, and all the necessary expertise, know-how and equipment is present and running to perform the proposed measurements and development, therefore we don't see any problems with feasibility. However, it is very important that the senior researcher for this project will be experienced in mass spectrometry.

4. RELEVANTIE VOOR DE PRAKTIJK:

SHORT-RUN benefits:
In the first year of the project, the multivitamin assay will be developed and micronutrient plasma levels of samples stored in the biobank of COVID-19 patients will be determined. These results will be immediately applicable to daily practice, because we will know whether the current practice of supplementation during the first 5 days with Cernevit and Supliven is sufficient to normalize these concentrations in the COVID-19 patients, or whether we should increase and/or prolong the supplementation. In the second part of the project we will perform a prospective study in which we will use the multi-vitamin assay together with trace element analysis in order to perform *personalized, daily adjustment* of micronutrient levels based on profiling results. This project will provide an important tool for assessment and optimization of the micronutrient status of our patients, crucial for their immune defense.
LONG-RUN benefits:
The results of this project can be used to start as a new (second) project an RCT comparing *targeted detection-correction* of multiple micronutrients with standard of care. This RCT will be embedded in the REMAP-CAP trial. Amsterdam UMC, location VUmc, will participate in the vitamin C domain of the REMAP-CAP trial. So, all the necessary logistic support will already be available. In this RCT we will apply AutoKinetics (a clinical decision support system which is already being used for antibiotic dosing) for micronutrient dosing. By feeding electronic health record patient data and the daily available micronutrient plasma concentrations into pharmacokinetic models, a tailored dosing advice will be provided at the bedside in real time. Optimization of micronutrient plasma levels may be of great benefit for the patients and could lead to a faster recovery of organ dysfunction, a decrease in mortality and earlier rehabilitation after ICU admission.
The ability to develop and apply this state-of-the-art multiparameter vitamin assay, the already existing participation in REMAP-CAP and the existing knowledge and experience of Autokinetics makes our University Hospital a unique place and well-qualified for this project. The presence of all the necessary collaborating departments in our University Hospital increases the chances of a successful project tremendously. The results will also be relevant for SARS-COV-3, the next influenza or every other new pandemic. Moreover, the results will also be applicable for the general ICU-population. Since REMAP-CAP is a large international trial, the results will be easily widespread internationally. Once developed, the multiparameter vitamin assay could be spread out to other hospitals as well. To the best of our knowledge, there is currently no similar project running elsewhere.

5. DEELNAME VAN DE STAKEHOLDER(S) (e.g. patiënten, zorgprofessionals, etc.):

- Collaboration of the department Clinical Chemistry (Prof. dr. R. de Jonge) and the OncoProteomics Laboratory (Prof. dr. C.R. Jimenez) with the department Intensive Care (dr. (10)(2e))
- REMAP-CAP ((10)(2e) :
- Autokinetics ((10)(2e)
- Prof. dr. H. Oudemans – van Straaten (Emeritus Professor Amsterdam UMC, location VUmc)
- Critical care Nutrition expert Dr. (10)(2e)
- (10)(2e) (initiator and coordinator of the 'Sepsis en daarna' patient platform and support group)

6. LITERATUURREFERENTIES (optioneel):

1. Romano, L., et al. Eur Rev Med Pharmacol Sci, 2020. **24**(7): p. 4035-4039.
2. Zhang, L., et al. J Med Virol, 2020. **92**(5): p. 479-490.
3. Calder, P.C., et al. Nutrients, 2020. **12**(4).
4. Li, T., et al. Eur J Clin Nutr, 2020.
5. (10)(2e) . et al. Critical Care, 2015: p. 445-458.
6. (10)(2e) ., D. et al. Nutrition, 2011. **27**(7-8): p. 745-58.