Abstract

Due to the lack of the sufficient quantity of the information about the loss of vitamins and trace elements during the continuous renal replacement therapy, the purpose of this thesis was to determine the pathways for the loss of these compounds as a result of the treatment. Among the specialists, opinions about the causes of vitamin deficiency and the trace elements disturbances level in patients are divided. Major hypotheses suggest to remove these compounds with ultrafiltrate or adsorption on the hemofilter surface or insufficient vitamin suppling in the diet.

Considering all challenges mentioned above, the purpose of this thesis was to develop a procedure for the: concentration, isolation and quantification analysis of water-soluble vitamins (vitamin C and B-group vitamins) and trace elements (Zn, Cu, Cr, Cd, Pb, Ni) from ultrafiltrates of patients who undergoes continuous renal replacement therapy and its practical implementation into clinical usage. It was established that the developed procedure will be implemented for the clinical usage at the Intensive Care Unit of the Medical University of Gdańsk.

Firstly, the possibility of sequestration of the vitamins on the haemofilter surface was checked as a cause of their removal from the body. There has been no adsorption of vitamins on hemofilters used in continuous renal replacement therapy, indicating the possibility of removing them together with ultra-filtrate. For this reason, during the further stage of research, methods of isolation and determination of vitamins and selected trace elements in biological samples were developed.

As a result of the studies, a procedure for the determination of water-soluble vitamins and trace elements has been developed and validated. In addition, a series of clinical specimens was tested. Water-soluble vitamins and trace elements (such as: cadmium, lead, nickel, chromium, copper, zinc) have been found in ultrafiltrates.

In the course of the research, the analysis of water-soluble vitamins by HPLC-UV/Vis and LC-MS / MS was optimized. For the purification of ultrafiltrate specimens, the suitability of Amberlite XAD 2, DEAE Sephadex ion exchange resins and mixtures of these resins

(2:1, w/w), SPE technique and reverse osmosis were tested. The research has shown a high purification capacity with DEAE Sephadex resin. The methodology of isolation and determination of 12 vitamins in ultrafiltrates using ion-exchange resin for purification of samples and LC-MS/MS as analysis techniques was validated. The above methodology was used to analyze ultrafiltrate samples from patients subjected to continuous hemodiafiltration. In the ultrafiltrates detected most of vitamin: B1, B2, B3 in the nicotinic

acid amide form, B5, B7 and two vitamin B6 forms (pyridoxal and pyridoxamine). Vitamin B12 was not detected, and the concentration of vitamins B9, B6 in the pyridoxine form and B3 in the nicotinic acid form oscillated at the LOQ level or were below the limit of determination.

Several preparation methods of ultrafiltrate specimens for the determination of trace elements have been tested. For mineralization of biological samples MAE technique with concentrated nitric acid was used and ASA technique was used to analyze trace elements. The developed MAE/ASA methodology has been validated. It can be successfully used to determine selected trace elements in ultrafiltrate samples. The results of this study indicate the removal of trace elements such as: cadmium, lead, chromium, zinc and nickel with the ultrafiltrate obtained from the applied therapy. In most samples the concentration of copper didn't exceed 0.1 μg/l.

The results of this study clearly confirm removal of certain water-soluble vitamins and trace elements together with ultra-filtrate. It suggests the need to monitor patients who undergoes renal replacement therapy.