Review of: "Prediction of survival odds in COVID-19 by zinc, age and selenoprotein P as composite biomarker"

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Selenium (Se) is an essential trace element that exerts its biological roles through up to 25 different selenoproteins in human being. In animal models, expression of some selenoproteins (so-called housekeeping genes) can be impressed for others (so-called stress-responsive genes) during limitation of Se intake, following with development of a selenoprotein hierarchy. That is to say, when dietary Se intake is insufficient for full expression of 25 selenoproteins in human body, some selenoprotein genes are supposed to be downregulated so that other selenoproteins can be produced fully as much as possible. Housekeeping Genes for the intracellular selenoproteins, glutathione peroxidase 3 (GPx4) and thioredoxin reduxtase 1 (TrxR1) which often ranks near the top of the selenoprotein hierarchy expresses stably, are not suitable to be used as biomarkers for the status of selenium. Obviously, those extracellular selenoproteins, including Selenoprotein P (Sepp1, encoded by the *SELENOP* gene) and glutathione peroxidase 3 (GPx3), are believed to be the better biomarkers for the status of selenium in human being. Sepp1 is mainly synthesized by hepatic cells in the liver and then released into plasma, is a glycoprotein with up to 10 selenocysteine (SeCys or Sec) residues which functions as the primary transporter of Se which is essential for proper distribution and efficient utilization of Se among extrahepatic tissues of human body, preferentially in providing the brain and testes with adequate Se especially under conditions of limited Se in diets. Selenoprotein P (Sepp1) is responsible for selenium homeostasis. Uptake and utilization of Se from Sepp1 in extrahepatic tissues involves two members of the low-density lipoprotein receptor (LDLR) superfamily, apolipoprotein E receptor-2 (apoER2) and megalin to forms and facilitate endocytosis, respectively.

ApoER2, a transmembrane protein with 963 amino acids and a molecular weight of 105 kDa, belongs to the classic LDLR family. ApoER2 is the major receptor for Sepp1 in mammals, and is preferentially expressed in the brain, placenta, and testis. ApoER2 facilitates Sepp1 endocytosis and Se supply to the cells. At the blood-brain barrier as well as within the brain, for instance, ApoER2 usually functions as Sepp1 receptor to keep an extra/intracellular Se pool for the synthesis of some important selenoproteins, such as glutathione peroxidase 4 (GPx4). In the brain, GPx4 was recently found to be required to prevent the H_2O_2-induced ferroptosis, an iron-dependent, nonapoptotic cell death. Animal models with a deletion of Sepp1 (Sepp1(-/-)) or a deletion of its receptor apoER2 (apoER2(-/-)) can develop severe dysfunction of the

central nervous system and infertility in male animals, especially when fed low-Se diet.

Megalin, a single transmembrane glycoprotein consisting of 4655 amino acids with a molecular weight of 600 kDa and is well known for its presence on the apical surface of renal proximal convoluted tubule (PCT) cells and its role in reclaiming proteins and other ligands from the glomerular filtrate in kidney. Megalin, unlike apolipoprotein E receptor-2 (apoER2), is a distant member of the LDLR family, also mediates the endocytosis of filtered Sepp1 forms. Such uptake is supposed to provide Se for synthesis of GPx3, the only extracellular one of GPxs family, secreted by PCT cells in the kidney, and then released into plasma, and to prevent loss of Sepp1 forms in the urine, too. In a word, the enzymatic activity of plasma GPx3 is generally regulated by a level of plasma Sepp1 in human body.

Because of limited accessible tissues in human studies, a short list of biomarkers of Se function is available: the enzymatic activity of erythrocyte GPx1, the first selenoezyme discovered, can be repeatedly found in the earliest human studies; the enzymatic activity of plasma/serum GPx3, which accounts for 25% of Se in plasma; plasma/serum Sepp1concentration, which comprises 70% of Se in plasma. Among these biomarkers, plasma Sepp1 concentration appears to be the best functional biomarker for assessing optimal expression of all selenoproteins for human studies implied from a study taken in China: maximal expression of Sepp1 may occur at a higher level of Se status than maximal expression of GPx3 in Se-deficient Chinese adults with selenomethionine (SeMet) supplementation. Based on this, the Chinese Nutrition Society decided to revise the reference intake of dietary selenium for Chinese adult residents from 50μg per day to 60μg per day in 2013. However, the clinical importance of plasma Sepp1 in human diseases has not been well defined.

Dr. Raban Arved Heller suggested recently in “Prediction of survival odds in COVID-19 by zinc, age and selenoprotein P as composite biomarker” that serum Sepp1 concentration can be used alone or together with serum zinc concentration as composite biomarker for prediction of survival odds in COVID-19, because of higher prevalence of deficit of serum Sepp1 alone or together with deficit of serum zinc was often reported in COVID-19 patients[1]. Selenium is an essential trace element that plays a vital role in development and a large amount of biological functions including immune barrier and immune response. The normal immune system relies on adequate intake of Se from diets alone or together with supplements and depends on optimal expression of several crucial selenoproteins directly. The most characterized selenoproteins are those antioxidant selenoenzymes, such as GPxs and TrxRs, including Sepp1, are well-known for their relationships with immune functions (specially cellular immune functions, usually including T cells, NK cells and monocytes) in mammals and selenoprotein K (encoded by the *SELENOK* gene) is well characterized among non-enzymatic selenoproteins. Most of all, Se deficiency can drive great changes in a viral genome, and then this genetic modification can permit these viruses (well-known as coxsackievirus B3 (CVB3), HIV and influenza viruses) mutate to highly pathogenic strains and avoid the immune response of the body[2]. We also assume that this correlation also applies to SARS-CoV-2. The pandemic of coronavirus disease (covid-19) is the cause of current high mortality around the world, caused by SARS-CoV-2 infection and enhanced by its toxic mutant-δ stain, among the elderly and people with

comorbidities[3]. It is reasonable to believe that insufficient Se intake will make the disease more serious and complex. In principle, yes, we agree with Dr. Raban Arved Heller on that the analysis of biomarkers (such as plasma Se concentration, the enzymatic activity of plasma GPx3 and plasma Sepp1 concentration) for Se status in COVID patients can add useful information for predicting their survival accurately.

However, there are several problems to be further clarified when we judge whether plasma Sepp1 is an optimal biomarker of Se nutritional status in human being.

At first, can false Sepp1 (through the substitution of SeCys by Cys in Sepp1) synthesized in the human liver be secreted into the blood and the substitution ratio of SeCys/Cys in plasma Sepp1 shift with the change of total Se intake within a certain range? We already know that Se, sulfur (S) and oxygen (O) belong to the same group of elements in the Periodic Table of Elements. It is not difficult to understand that the only difference between SeCys, cysteine (Cys) and serine (Ser) is that there is only one element (Se, S and O in turn) with similar chemical molecular formula and spatial three-dimensional structure, and it is not surprising that people come to realize that the substitution of SeCys by Cys in selenoproteins is not a rare phenomenon in the whole biological world. The only surprise is the mechanism by which SeCys is replaced by Cys in the synthesis of false selenoproteins in mammals. Like SeCys as SeCys-tRNA$^{[Ser]SeCys}$, Cys can only use Ser and its tRNA to de novo synthesize Cys-tRNA$^{[Ser]Sec}$ by a series of enzymatic reactions and Cys also needs to utilize the same codon (UGA, one of common termination codons(UAA, UAG and UGA)) specific for SeCys instead of its own corresponding common codons during its incorporation process in false selenoproteins. Nowadays, false selenoproteins, including false Sepp1, have already been found in Se-deficient animal models and also in a small number of human liver tissues[4]. Based on the effects of Ser and S-containing amino acids on the synthesis of true and false selenoproteins[5], we hypothesized that the gradual disappearance of Keshan disease, which was once a large-scale epidemic among local residents in Se-deficient areas in China, may include the current greatly improved dietary protein intake of local residents, which is helpful for the synthesis of false selenoproteins to substitute the biological and physiological roles of true selenoproteins, and then a physiological adaptation to low-Se intake in residents occurs in these areas in China[6]. This physiological adaptation mechanism to dietary low-Se intakes might also occur in those residents with Se-deficient or low-Se diets in other countries around the world where there wasn’t a prevalence of Keshan disease, partly because of their diets rich in animal tissues with a higher level of dietary protein, and quite different from the traditional Chinese diet, which is dominated by plant food.

The second one is whether the persistent saturation level of plasma Sepp1 can represent the best/optimal selenium nutritional state of human body. In 2004, the first study reported the negative impact of overexpression of selenoproteins on physiological functions came from a transgenic animal model[7] and did not attract due attention. In this study, insulin resistance (IR), often regarded as a hallmark of type 2 diabetes, was unexpectedly found to be developed in GPx1-overexpressing male mice[7]. Until 2007, based on a reanalysis of data collected from a small cancer prevention cohort (NPC) in the United States

and Canada, the positive relationship between Se supplementation (200μg Se/day as Se-enriched yeast) and the increasing risk of type 2 diabetes(T2D) was firstly discovered in human being[8]. And then this positive relationship was soon confirmed in another great cancer prevention cohort (SELECT) in North America again in 2009[9]. In SELECT, Se supplementation was administrated as 200μg Se/day as SeMet instead of Se-enriched yeast[9]. Then in 2010, the administration of purified Sepp1 in normal mice fed with conventional diet was found to impair insulin signing and disturb glucose metabolism and then develop IR too[10], and the metabolic roles of Sepp1 were partly due to the impression of adenosine monophosphate-activated protein kinase (AMPK). In the same study, level of Sepp1 mRNA expression was also reported to be correlated with IR in human liver samples (from 5 patients with T2D and 5 normal subjects). Later in 2017, a neutralizing monoclonal antibody against human sepp1 and a polyclonal antibody against the mouse selenoprotein P were developed by the same research group to antagonize the IR effect in mice administrated with human Sepp1 or in mouse models of diabetes successfully[11]. Based on above experimental evidences and a large number of cross sectional survey data from different countries and races, a systematic review and evidence-based analysis showed recently that the daily intake of selenium reached more than 80μg (both plasma Sepp1 concentration and plasma GPx3 enzymatic activity are supposed to persist at a saturation plateau) might give rise to the risk of T2D[12].

Finally, the current methods(ELISA kits, for instance) used for the measurement of human plasma/serum Sepp1 mainly analyze its total concentration, and there is no method suitable for the intact full-length Sepp1, let alone the method to distinguish true and false Sepp1, up to now[13]. Unlike other selenoproteins, mammal selenoprotein P is a unique glycoprotein containing multiple SeCys residues. For example, there are 25 SeCys residues on a sepp1 polypeptide chain in human. Plasma Sepp1 usually appears in the form of multiple isoforms and truncated species. Accurate quantification of plasma true sepp1 is indeed a great challenge, depending on the availability of specific primary standards and reference methods. In principle, the true Sepp1 from human plasma as a glycoprotein in the nature form of multiple isoforms and truncated species can be separated and purified through specific human antibodies developed by bioengineering technology to be used as specific primary standards as well as HPLC-ICP-MS will be used as one of reference methods.

In summary, although Sepp1 is synthesized mainly by the liver and then secreted into plasma to maintain the selenium homeostasis in human, there might be a physiological adaptation mechanism that SeCys can be replaced by Cys to synthesize false sepp1 when the intake of Se is insufficient. However, the current analysis methods cannot distinguish true and false sepp1, resulting in that the total concentration of plasma sepp1 does not always sensitively and accurately reflect the real nutritional status of Se in the body. In addition, evidences from animal models, population cross-sectional surveys and population cohorts showed that persistent high expression of selenoproteins (such as GPx1 and Sepp1) could increase the risk of T2D. Therefore, it is time for developing a new method that can effectively distinguish true Sepp1 from false one and sensitively and accurately determine the content of true Sepp1 in plasma, so as to clearly find out whether the content of true sepp1 in plasma is suitable to be used as a biomarker of

human Se requirement or an auxiliary index in diagnosis of related diseases including T2D.

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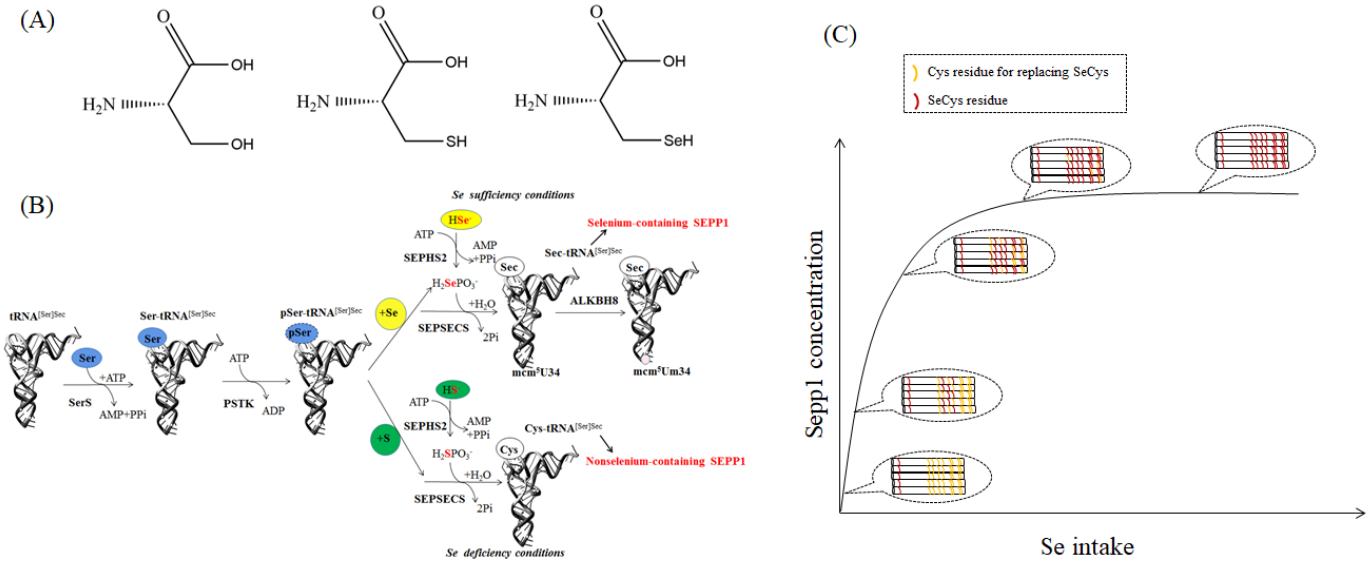

Figure 1 (A) The chemical structures of l-serine, l-cysteine, and l-selenocysteine. (B) The replacement of Sec or Cys by a de novo synthesis dependent on a unique Ser-tRNA. (C) the numbers of SeCys residues in Sepp1 supposed to vary with the Se intake.