Should We Search for Early Brain Disease Biomarkers in Urine

Abstract

Neurons are exquisite sensitive to alterations. The brain has a high metabolic rate; thus, it requires rapid mobilization of nutrients and clearance of metabolites to maintain constant homeostasis in the environment around the neurons and glia. We discuss the normal ranges of metabolic products in the cerebrospinal fluid (CSF), blood and urine and the direction of nutrient supplement from blood to CSF. Furthermore, we summarize the clearance pathway of the metabolic products from CSF to urine. Body fluids, which take nutrients from others, discard metabolites to others and have a narrower normal range of metabolic products, have a higher homeostasis status. We suggest the cerebrospinal fluid has higher homeostatic priority than blood. Unlike CS For blood, which is kept stable by homeostatic mechanisms in which early changes associated with disease were removed, urine is an ideal source of changes that can reflect conditions of the brain, especially chronic conditions.

Keywords: Homeostatic ranking; Cerebrospinal fluid; Interstitial fluid; Cerebral wastes; Biomarkers; Homeostasis; Blood; Urine

Yanying Ni[1] and Youhe Gao[2]*

[1]*Chinese Academy of Medical Sciences/Peking Union Medical College, China*
[2]*Departments of Biochemistry and Molecular Biology, Beijing Normal University, China*

***Corresponding author:** Youhe Gao, Department of Biochemistry and Molecular Biology, Beijing Normal University, Gene Engineering and Biotechnology Beijing Key Laboratory, Beijing, 100875, PR. of China, Tel: 86-10-58804382; Email: gaoyouhe@bnu.edu.cn; ORCID Id: orcid.org/0000-0001-7159-2558

Abbreviations: CSF: Cerebrospinal fluid; CNS: Central nervous system; BBB: Blood brain barrier

The Importance of Homeostatic Ranking of CSF, Blood and Urine

Biomarkers can be used to measure changes associated with physiological or path physiological processes. Thus, the most fundamental feature of a biomarker is the 'change' between healthy and disease states (Figure 1). We believe that urine is more optimal for early biomarker detection because it accumulates many changes [1]. The cerebrospinal fluid (CSF), which is associated with the central nervous system (CNS), will rapidly discard the changes to remain stable. Blood, which circulates throughout the body to interact with organs, also has a relatively dynamic steady state. In the early stage of disease, it is difficult to detect early biomarkers under the condition of a compensatory steady state. During the dyshomeostasis stage, in which the stacking velocity of metabolite products is greater than the clearance speed, the detectable changes are probably not early biomarkers [2]. By contrast, urine accumulates changes in the blood and may tolerate and even magnify changes without causing harm to the body [3-5]. When the CSF and blood are in homeostasis in the early stage, urine, which collects bodily waste, reflects the early changes that are occurring inside of the body [6-8].

The evaluation principle of homeostatic priority

The body fluid which has a narrower normal range has a higher homeostasis status; the body fluid which takes nutrients from others has higher homeostasis ranking; the body fluid which discards metabolite products to others has higher homeostasis ranking. Thus, we suggest that the brain has a higher homeostatic priority than blood (Figure 2).
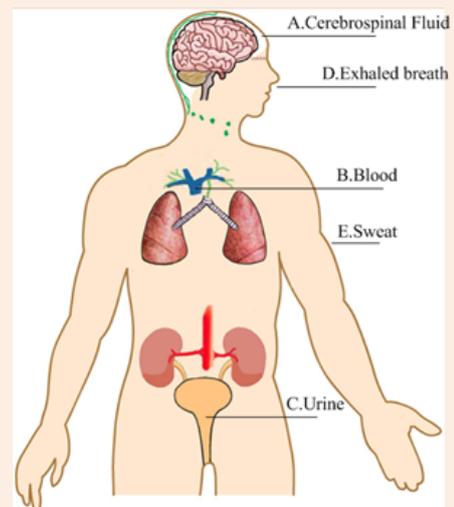

Figure 1: Flow Directions of the Cerebral Wastes Considered. There are several places for us to capture biomarker.
A. The CSF covers biomarkers that are associated with brain intimately.
B. The blood transport a large number of metabolite information produced by organs and tissues.
C. The urine removes unwanted substances which mainly are inherited from upper fluids, such as urea, excess water and other changes.
D. The air we exhaled out contains water, CO2 and volatile organic compounds (VOCs).
E. The skin provides a surface for small amounts of water and salt to move out of the body. With the flow direction of metabolite wastes from brain to blood, eventually to urine, the homeostasis is gradual decline.
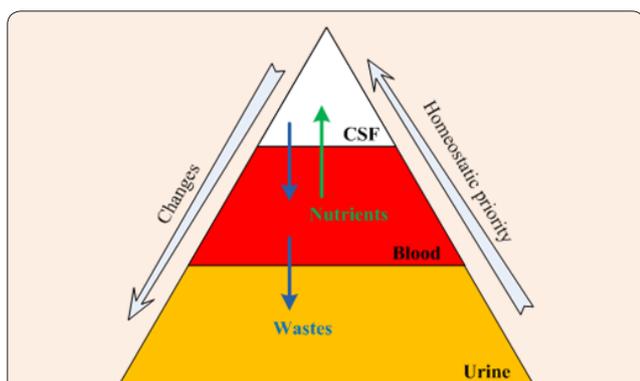F. Lymphatic system provides a pathway for wastes from brain to blood.

Figure 2: The Homeostasis Ranking of CSF, Blood, Urine.

A. The blood provides nutrients to CSF.

B. The cerebral wastes will be excluded to blood in certain form, some will be excluded to urine.

C. With the discharge direction of wastes, the homeostasis is declined, the CSF has a homeostatic priority than blood, the blood has to make associate adjustment to maintain the homeostatic priority of brain, and the urine doesn't have homeostasis.

D. With the discharge direction of wastes, the CSF and the blood remove changes, the urine collects most changes.

The normal range of CSF, blood, and urine

The CNS is the most critical and sensitive system in the human body. To maintain brain homeostasis, the blood supplies nutrients to the brain, and the blood brain barrier (BBB) restricts potentially harmful molecules that are present in blood [9,10]. The BBB, which is formed by endothelial tight junctions, pericytes, perivascular astrocytes, and basement membrane in the vasculature [11] regulates ion balance in the brain and facilitates the transportation of nutrients to the brain [11] Proper neuronal function necessitates a highly regulated extracellular homeostasis in which the concentrations of ions and pH must be maintained within very narrow ranges.

Glucose, which provides the energy to support all activities in the body, is modulated according to the body's metabolism. The normal range of glucose in the CSF is narrower than in the blood [12,13] while serum glucose fluctuates throughout the day. When the blood glucose concentration is low, such as when food intake is limited or in disease, the glucose level in the cerebrospinal fluid does not rapidly change. Furthermore, the reabsorption function of the kidneys ensures that sugars are not readily excreted through the urine [14]. When blood sugar increase, (for instance, during intravenous glucose injection), the blood glucose concentration changes fast, while the CSF glucose changes more slowly [15]. Glucose is usually regarded as the primary energy source for body tissues. Lactate, which is provided by glial cells to neurons [16], may be another energy source for neurons in the brain [17]. Lactate is important for the brain metabolism in the early stages of development in prenatal and early postnatal subjects. In these stages, lactate is abundant in the CSF, and its levels fluctuate more in the CSF than in blood [17,18] The CSF has a higher priority based on its metabolic requirements, and despite wide

fluctuations between meals or the occasional consumption of meals, blood glucose levels tend to remain within a narrow range. However, the glucose levels in urine are dynamic to maintain the homeostasis of the whole body.

Acid-base balance is a premise for internal homeostasis, especially for the brain, in which neurons and glia are exquisitely sensitive to changes. The normal acid-base range in the CSF is narrower than in blood [19,20]. With chronic acid-base disturbances, the pH fluctuation in the CSF is also narrower than in blood [20]. The CSF pH is stable. In past studies, a transient increase in pCO_2 in the artery did not alter CSF pCO_2 [21,22] after an intravenous infusion of hydrochloric acid over approximately 24h followed with isotonic sodium bicarbonate, the pH in the CSF was not significantly different [23]. Urine that contains various of changes from kidney which transfer the acid into urine and reabsorb HCO3- consumed in blood has a broad range of pH[24] the range of 4.8~7.4 has suggest that there are a lot of changes in urine and the CNS is in a stable environment all the time, even in acid-base disturbance[25,26].

In ion tests of the CSF and blood (Table1), most ions ranges of the CSF are narrower than of blood [12,27,28] except for chloride, which is narrower in blood than the CSF [29]. The osmolality and the sodium ions ranges of the CSF and blood are approximately similar [28] while the sodium and potassium levels in urine tend to fluctuate widely over the course of a day [29].With renal injury, electrolyte disturbances can occur. Because of kidney dysfunction, both metabolic wastes and ions, such as serum urea nitrogen, creatinine and potassium, will accumulate and cause harm to the body. The cumulative levels of harmful electrolytes in cerebrospinal fluid are lower than in blood.

Supply of nutrition from blood to brain

It is well known that the blood, which provides nutrients to the whole body, also provides adequate nutrients to the brain to ensure optimal function of the CNS [30]. Adequate supply of glycogen has important implications for the functioning of the brain, especially the cooperation between astrocytes and neurons [31]. As for glucose consumption, the central nervous system has absolute priority. Many nutritional solutes in blood can enter the central nervous system. Small molecules, such as amino acids, hormones, water and fat-soluble molecules, (e.g., oxygen), can easily cross the BBB. Specific glucose transporters (GLUTs) can transport special nutrients, such as glucose, cross the BBB [32]. In addition to the primary energy source, lactate, can also be transported into the brain [31]. Some substances, such as organic anions that can't easily cross the BBB, are mainly carried by transporters. For the transportation of large amino acids and polypeptides, OATPs and LAT1 play important roles [33] and PEPT2 in choroid plexus tissue quickly transports di peptides into the brain [34]. Members of the OAT family transport a wide range of drugs, such as aspirin, ibuprofen, various antibiotics, and pesticides [33].

It is well-accepted that blood provides nutrients to the brain; thus we focus minimal attention to cerebral nutrition directions in this review. Because the BBB restricts the removal of cerebral products, we focus on the discharge direction of metabolic products.

Table 1: The normal range of CSF and blood.

Substance	Unit	Cerebrospinal Fluid	Fluctuating Value	Ref.	Blood	Fluctuating value	Ref.
Glucose	mmol/L	2.8～4.4	1.6	[12]	3.5～5.5	2	Fasting [13]
Lactate levels	mmol/L	1.1～2.4	1.3	[12]	0.5～1.6	1.1	Arterial [18]
pH	no unit	7.28～7.32	0.04	[12]	7.35～7.45	0.1	[15]
Metabolic acid-base imbalance	no unit	7.315～7.337	0.022	[19]	7.350～7.523	0.173	[19]
Respiratory acid-base imbalance	no unit	7.314～7.336	0.022	[19]	7.382～7.485	0.103	[19]
PCO2	mmHg	44～50	6	[19]	35～45	10	Arterial [28]
PO2	mmHg	40～44	4	[12]	75～100	25	Arterial [28]
Osmolality	mmol/L	280～300	20	[12]	280～296	16	[114]
Potassium(K)	mmol/L	2.6～3.0	0.4	[12]	3.5～5.0	1.5	[115]
Sodium (Na)	mmol/L	135～150	15	[12]	135～147	12	[115]
Chloride(Cl)	mmol/L	115～130	15	[12]	100～110	10	[116]
Calcium(Ca)	mmol/L	1.0～1.4	0.4	[12]	2.1～2.8	0.7	[116]
Magnesium (Mg)	mmol/L	1.2～1.5	0.3	[12]	1.5 ～2.0	0.5	[114]
Urea	mmol/L	3.0～6.5	3.5	[12]	3.0～7.0	4	[117]

Removal of cerebral wastes from brain to blood

Extracellular homeostasis requires the ability to rapidly clear metabolic products from the brain [34]. In addition to transporting biologically active substances to the CNS [35] cerebral fluids, which are indispensable for brain homeostasis, remove metabolites from the parenchyma through several pathways (Figure 3).
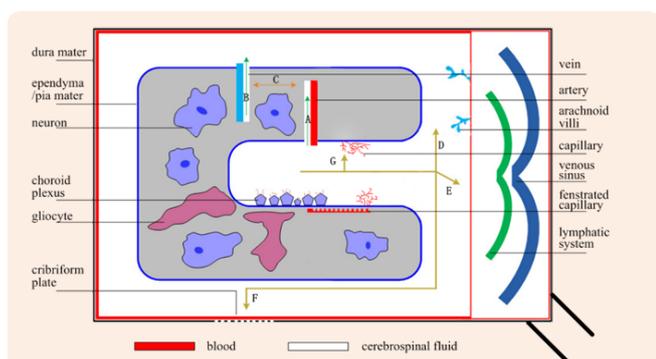
Figure 3: Fluid Movements of the whole brain considered.
  A. Fluid components can move through the parenchyma along par arterial space.
  B. The ISF outflows from parenchyma via para venous space.
  C. The exchanges between ISF and CSF.
  D. The CSF outflows across the arachnoids villi leading to the dural venous sinuses.
  E. The CSF can be excluded along dural lymphcatic system.
  F. The CSF can be removed via the olfactory nerve leading to the cribriform plate.
  G. CSF can be abruption by capillaries everywhere in ventricles.

Wastes from the parenchyma to CSF

Neurons are surrounded by interstitial fluid (ISF), which carries metabolite wastes from cells to maintain constant homeostasis of the CNS [36]. Small compounds can readily pass into the CSF; however, it is impossible for larger molecules located deep within the brain parenchyma to easily move into the CSF [36]. Some studies have suggested that perivascular spaces may remove larger wastes from the parenchyma into the CSF [37]. Physical connections between the CSF and perivascular spaces around the brain vasculature [38,39] and between ISF and perivascular spaces suggest that extracellular markers that are injected into the parenchyma will be cleared from the brain [40,41] Thus, the changes happened in brain will be removed from brain parenchyma in a certain form.

It was thought that substances slowly diffuse into the CSF [43] However, albumin requires more than 100 hours to diffuse through 1 cm of brain tissue [42] which conflicts with the two-photon imagines that show thatthe CSF is exchanged rapidly with the ISF in the brain [43]. In one study solutes; varying in size from 4,000 to 69,000 Dalton; were injected directly into the brain and left the brain at similar rates, suggesting that convective loss was a major pathway of solute removal from the brain rather than diffusion [44,45].

Metabolic products, including small changes and larger wastes, are removed from the parenchyma and move into the CSF [46-48]. The perivascular spaces existing between the walls of veins and astrocyte end feets are known as the lymphatic system [49,50]. Interestingly mice lacking the water channel aquaporin-4 in astrocytes [51,52] exhibit a reduction in interstitial solute clearance [53] suggesting that the efflux is supported by the water

channel aquaporin-4[54] Partial ligation of the brachiocephalic artery prevents therapid paravascula refflux of tracers, suggesting that artery pulsation improves the clearance of potential harmful products, including amyloid β [55,56]. During the movement of cerebral fluids, other physiological factors potentially influence the clearance of cerebral products, such as sleep [57,58] body posture [59].

The importance of understanding the mechanisms of brain waste efflux from the parenchyma is highlighted in neurodegenerative diseases, characterized by the pathological accumulation of misfolded proteins in the interstitial space, including β-amyloidal (Aβ) [60,61] and tau [62] Aβ is thought to be a pathogenic peptide in Alzheimer's disease. Endogenous substances, such as Aβ and tau, are cleared in the perivascular spaces to maintain homeostasis of the brain [53,63].

Wastes from the CSF to blood

The BBB provides constant protection for the CNS, while restricting the removal of metabolic products that will harm neural tissue [64] The CSF can transport products from the brain into the bloodstream via capillaries or the lymphatic system, which play a major role in maintaining the electrolytic and acid-base balance of the CNS (Table 2).

The CSF, formed mainly in the ventricles of the brain, flows through the cerebral ventricles into the subarachnoid spaces; then the CSF moves into the bloodstream by arachnoids villi and the cerebral changes are moved to the blood [65] This classical route is efficient for the excretion of water and small compounds into the bloodstream, while larger compounds are unlikely to be transferred to blood through this route [66].

It is generally accepted that the CNS, which required rapid clearance of ISF and solutes, does not contain lymphatic vessels. However, vessels expressing lymphatic endothelial cell markers in the dura suggest that there may be lymphatic structure here [67,69]. Larger molecules, such basal bumin can be measured continuously in cerebral perivascular spaces, dura lymphatic system and lymph nodes when injected into the brain [70,75] suggesting that lymphatic structures allow macromolecules to flow from the brain to the blood [76,79]. Aplasia of dura lymphatic vessels will reduce macromolecules clearance [80]. These findings suggest that non-cardiovascular structures play an integral role in transporting wastes from the brain, including larger molecules [81]. The functional lymphatic system also provides a way for the outflow of immune cells, which mainly accounts for the slower immune reactions in the brain [82]. The transportation of APCs by the dura mater lymphatic system may play a central role in experimental autoimmune encephalomyelitis (EAE), in which T cells specific to CNS antigens traffic to the brain and result in paralysis [83]. To our regret, the process of immune cell activations in deep cervical lymph nodes after injury remains poorly understood [84].

In addition to the above pathways of CSF drainage, olfactory bulbs are another efflux pathway of the CSF [85-89]. Tracers are located not only in the subarachnoid compartment, but also within the olfactory sub mucosa. Furthermore, the tracers are situated within an extensive network of lymphatic vessels in the nasal sub mucosa, which suggests that the way through the cribriform plate may take the CSF from the brain to the lymphatic system, which is associated with the sub mucosa of the olfactory and respiratory

epithelium [90,92].The cribriform plate plays a major role in the efflux of immune cells, such as CD4T, dendritic cells (DCs) and monocytes, from the brain to the peripheral lymphatic system [93,94].

The CSF absorption takes places not only through arachnoid granulations in the subarachnoid space or lymphatic system, but also through capillaries inside the brain ventricles in other words, the CSF disappears and is reabsorbed everywhere in the cerebral system [95,96] There is no need for directed CSF circulation from the choroid plexus (CP) to the arachnoid villi; instead, CSF production and absorption occurs at the level of the capillaries and is not limited to the location of the capillaries [97,98].

The high sensitivity of neural cells to toxic substances demands that the brain remove products quickly and efficiently, which is the premise for the homeostasis of the brain [53]. Small molecules, hydrophobic compounds, and larger compounds can be excluded from the brain [36,99] though the cerebral changes may be modified, degraded or have other transformations, it is reasonable for us to suggest that cerebral changes can move from the brain to the blood.

Clearance of wastes from blood to urine

In most issues and organs, metabolic products in the interstitial fluid are excreted into the local lymphatic system and eventually into the blood, preventing the accumulation of potentially toxic compounds that will harm the body [66].The blood transports metabolites, including cerebral metabolic products and peripheral metabolic wastes, to certain organs that will excrete them from the body, such as the lungs [100] skins [101], and kidneys [102]. The kidneys, which act as filters, are main excretory organs and play an important role in removing metabolic products from the blood into the urine [103]. The kidneys maintain electrolyte and acid-base balance when the body is not performing properly. The kidney can excrete small wastes, such as urate, urea, and toxins [104] and also some proteins into urine [105] while restricting substances that are necessary for maintaining normal homeostasis. Thus, the majority of products are discharged into the urine. Cerebral changes, including wastes or other metabolic information, will be excluded from the brain to the blood in a certain form. Some changes will be excluded from the blood to urine, which collects early metabolic information associated with the whole body [6,8].

Urine is an ideal place to search for early biomarkers of chronic brain diseases

The CSF has a greater homeostatic priority than blood, and changes in the CSF may happen later than in blood. Changes in urine occur more rapidly than in blood; thus, urine may be a more optimal environment for detection of earlier biomarkers of brain disease [106,107] which may have important clinical significance. Cerebral wastes are excreted from the brain to blood and; eventually to urine, which suggests that we should be able to detect early biomarkers of brain diseases in urine. Previous reviews have summarized several brain diseases, especially chronic diseases such as neuropsychiatric disorders [108] neurodegenerative diseases [109] and neuroendocrine neoplasm [93,110] that are difficult for early diagnosis. These diseases are mainly diagnosed based on a subjective symptom assessment. Thus far, no objective and effective measurement procedures are available [111].

Table 2: Crucial experiments proving larger molecules can be excluded from brain.

Study (year)	Subject	Injection site	Substance	Molecular Weight	Region	Ref.
Yamada K et al. [62]	mouse	left hippocampus	Aβ solution	4kDa	lymphatic pathways	[62]
Carare RO et al. [76]	mouse	striatum	ovalbumin (OVA) / fluorescent dextran	49/3-10kDa	lymphatic pathways	[76]
Kress BT et al. [66]	mouse	cisterna magna	Texas Red conjugated dextran	3kDa	lymphatic pathways	[61]
Schwalbe et al. [73]	dog, rabbit	CSF	Berlin blue	859	lymph nodes	[73]
Goldmann [85]	dog, rabbit	CSF	Trypan blue	960	lymph nodes olfactory nerves	[85]
Mortensen	dog	cisterna magna	Thorotrast	1626	lymph nodes	[74]
Yoffey et al. [86]	rabbit, monkey	lateral wall and septum of the nose	Higgin' India ink	--	lymph nodes olfactory nerves	[86]
Cserr et al. [72]	rat	caudate nucleus	polyethylene glycols/ dextran	70/4kDa	lymph nodes	[72]
Cserr et al. [44]	rat	caudate nucleus	serum albumin/	69 /4kDa	lymph nodes	[78]
McComb et al. [70]	rabbit	ventriclescisterna magna	RISA with dextran	>45kDa	olfactory nerve	[87]
Cserr et al. [79]	rabbit	caudate nucleus	radiolabelled albumin	>45kDa	deep cervical lymph	[79]
Brinker et al. [88]	cat dog monkey	CSF	dextran	70kDa	olfactory nerves NasalLymphatics	[88]
Boulton et al.[75]	rat	lateral ventricle	human serum albumin	>45kDa	lymph nodes	[75]
Zakharov et al. [89]	neonatal	cranial sub-arachnoid	Yellow Microfil	--	olfactory Nerves	[89]
	sheep				Nasal Lymphatic	
Ball KK et al. [60]	rat	inferior colliculus	Evans blue albumin	>45kDa	cervical lymph nodes	[60]
Liu H et al. [90]	rabbit	cisterna magna	Microfil	--	around the olfactory nerves and within lymphatic vessels	[90]
Kaminski et al. [37]	mouse	left entorhinal cortex	Monocytes	--	lymph nodes	[37]
Laman JD et al. [83]	animals / humans	subarachnoid space	antigen presenting cells	--	lymph nodes	[83]
Mathieu E et al. [81]	mouse	cisterna magna	Quantum dot 655	--	lymph nodes	[81]
Stern JN et al. [84]	five subjects with MS	the meninges, parenchyma	B cells	--	lymph nodes	[84]
Mohammad et al. [94]	mouse	lateral ventricle	CFSE-labeled immune cells	--	lymph nodes olfactory nerves	[94]
Plog BA et al. [77]	mouse	cerebral cortex cisterna magna	AlexaFluor-555-o-valbumin / 3H-dextran/ 14C-inulin	45/40/6kDa	lymph nodes	[77]
Louveau A et al. [66]	mouse	cisterna magna	Evans blue	--	lymph nodes	[67]
Daniel R Lu	multiple sclerosis	central nervous system	B cells	--	peripheral lymph nodes	[118]
Aspelund A [68]	mouse	brain parenchyma	ethylene glycol/ Alexa Fluor 488–conjugated OVA	20/45kDa	cervical lymph nodes	[68]

However, we can search for some early biomarkers that are associated with these brain diseases in urine, which can enhance assessments and predict treatment response. Urine analyses of control and disease samples in this review provide valuable clues for the diagnosis of these diseases [112] clinically applicable urine biomarkers of brain diseases may exist and should be explored in future diagnoses for complex brain diseases.

Concluding Remarks

The CSF has a homeostatic priority than blood, the urine which doesn't have steady state is an ideal place to search for early biomarkers for the complex chronic brain diseases, especially the early changes that cannot be captured in CSF or blood which is in homeostasis.

Funding & Competing Interests

References

1. Gao Y (2013) Urine-an untapped goldmine for biomarker discovery? Science China. Life sciences 56(12): 1145-1146.

2. WuJ, GaoY (2015) Physiological conditions can be reflected in human urine proteome and metabolome. Expert Rev Proteomics 12(6): 623-636.

3. Li M (2015) Urine reflection of changes in blood. Adv Exp Med Biol 845: 13-19.

4. Li M, Zhao M, Gao Y (2014) Changes of proteins induced by anticoagulants can be more sensitively detected in urine than in plasma. Sci China Life Sci 57(7): 649-656.

5. Gao Y (2014) Are urinary biomarkers from clinical studies biomarkers of disease or biomarkers of medicine. MOJ Proteomics Bioinform 1(5): 1.

6. Wang Y, Chen J, Chen L, Zheng P, Xu HB, et al. (2014) Urinary peptidomics identifies potential biomarkers for major depressive disorder. Psychiatry Res 217(1-2): 25-33.

7. Mackay CE, Roddick E, Barrick TR, Lloyd AJ, Roberts N, et al. (2010) Sex dependence of brain size and shape in bipolar disorder: an exploratory study. Bipolar Disord 12(3): 306-311.

8. Chen JJ, Liu Z, Fan SH, Yang DY, Zheng P, et al. (2014) Combined application of NMR- and GC-MS-based metabonomics yields a superior urinary biomarker panel for bipolar disorder. Sci Rep 4: 5855.

9. Walsh JT, Hendrix S, Boato F, Smirnov I, Zheng J, et al. (2015) MHCII-independent CD4+ T cells protect injured CNS neurons via IL-4. J Clin Invest 125(2): 699-714.

10. Bentivoglio M, Kristensson K (2014) Tryps and trips: cell trafficking across the 100-year-old blood-brain barrier. Trends Neurosci 37(6): 325-333.

11. Abbott NJ, Friedman A (2012) Overview and introduction: the blood-brain barrier in health and disease. Epilepsia 53(Suppl 6): 1-6.

12. Bondy DGP (2011) Pathology 425 Cerebrospinal Fluid [CSF], the Department of Pathology and Laboratory Medicine at the University of British Columbia, Canada.

13. Nessa A, Rahman SA, Hussain K (2016) Hyperinsulinemic Hypoglycemia - The Molecular Mechanisms. Front Endocrinol (Lausanne) 7: 29.

14. Verbeek MM, Leen WG, Willemsen MA, Slats D, Claassen JA, et al. (2016) Hourly analysis of cerebrospinal fluid glucose shows large diurnal fluctuations. J Cereb Blood Flow Metab 36(5): 899-902.

15. Li M, Zhao M, Gao Y (2015) Effect of transient blood glucose increases after oral glucose intake on the human urinary proteome. Proteomics Clin Appl 9(5-6): 618-622.

16. Panov A, Orynbayeva Z, Vavilin V, Lyakhovich V (2014) Fatty acids in energy metabolism of the central nervous system. BioMed Res Int 2014: 472459.

17. Schirmeier S, Matzat T, Klambt C (2016) Axon ensheathment and metabolic supply by glial cells in Drosophila. Brain res 1641(Pt A): 122-129.

18. Derived from mass values using molar mass of 90.08 g/mol.

19. McNamara J, Worthley LI (2011) Acid-base balance: part I. Physiology. Crit Care Resusc 3(3): 181-187.

20. Mitchell RA, Carman CT, Severinghaus JW, Richardson BW, Singer MM, et al. (1965) Stability of cerebrospinal fluid pH in chronic acid-base disturbances in blood. J Appl Physiol 20(3): 443-452.

21. Cowie J, Lambie AT, Robson JS (1962) The influence of extracorporeal dialysis on the acid-base composition of blood and cerebrospinal fluid. Clin Sci 23: 397-404.

22. Manfredi F (1962) Acid-base relations between serum and cerebrospinal fluid in man under normal and abnormal conditions. J Lab Clin Med 59: 128-136.

23. Abeysekara S, Zello GA, Lohmann KL, Alcorn J, Hamilton DL, et al. (2012) Infusion of sodium bicarbonate in experimentally induced metabolic acidosis does not provoke cerebrospinal fluid (CSF) acidosis in calves. Can J Vet Res 76(1): 16-22.

24. Kurtz I (2014) Molecular mechanisms and regulation of urinary acidification. Compr Physiol 4(4):1737-1774

25. Rose C, Parker A, Jefferson B, Cartmell E (2015) The Characterization of Feces and Urine: A Review of the Literature to Inform Advanced Treatment Technology. Crit Rev Environ Sci Technol 45(17): 1827-1879.

26. Pejcic M, Stojnev S, St efanovic V (2010) Urinary proteomics--a tool for biomarker discovery. Ren Fail 32(2): 259-268.

27. Rao DA, Le T, Bhushan V (2008) First Aid for the USMLE Step 1. McGraw-Hill Medical, New York, USA.

28. Normal Reference Range Table. In: Interactive Case Study Companion to Pathologic basis of disease. (Ed.^(Eds) (he University of Texas Southwestern Medical Center at Dallas.

29. Reference range list from Uppsala University Hospital ("Laborationslista"). (Ed.^(Eds) (April 22, 2008)

30. Ipata PL (2011) Origin, utilization, and recycling of nucleosides in the central nervous system. Adv Physiol Educ 35(4): 342-346.

31. Falkowska A, Gutowska I, Goschorska M, Nowacki P, Chlubek D, et al. (2015) Energy Metabolism of the Brain, Including the Cooperation between Astrocytes and Neurons, Especially in the

Context of Glycogen Metabolism. Int J Mol Sci 16(11): 25959-25981.

32. Nijland PG, Michailidou I, Witte ME, Mizee MR, vander Pol SM, et al. (2014) Cellular distribution of glucose and monocarboxylate transporters in human brain white matter and multiple sclerosis lesions. Glia 62(7): 1125-1141.

33. Saunders NR, Habgood MD, Mollgard K, Dziegielewska KM (2016) The biological significance of brain barrier mechanisms: help or hindrance in drug delivery to the central nervous system. F1000 Research 10: 5.

34. Warsi J, Hosseinzadeh Z, Elvira B Pelzl L, Shumilina E, et al. (2015) USP18 Sensitivity of Peptide Transporters PEPT1 and PEPT2. PloS One 10(6): e0129365.

35. Oreskovic D, Klarica M (2014) A new look at cerebrospinal fluid movement. Fluids Barriers CNS 11: 16

36. Verkman AS (2013) Diffusion in the extracellular space in brain and tumors. Physical Biol 10(4): 045003.

37. Kaminski M, Bechmann I, Pohland M, Glumm J, Nitsch R, et al. (2012) Migration of monocytes after intracerebral injection cortex lesion site. J leukoC biol 92(1): 31-39.

38. Hawkes CA, Sullivan PM, Hands S, Weller RO, Nicoll JA, et al. (2012) Disruption of arterial perivascular drainage of amyloid-beta from the brains of mice expressing the human APOE epsilon4 allele. PLoS One 7(7): e41636.

39. Ratner V, Zhu L, Kolesov I, Nedergaard M, Benveniste H, Tannenbaum A (2015) Optimal-mass-transfer-based estimation of glymphatic transport in living brain. Proc SPIE Int Soc Opt Eng 9413.

40. Carare RO, Hawkes CA, Jeffrey M, Kalaria RN, Weller RO (2013) Review: cerebral amyloid angiopathy, prion angiopathy, CADASIL and the spectrum of protein elimination failure angiopathies (PEFA) in neurodegenerative disease with a focus on therapy. Neuropathol App Neurobiol 39(6): 593-611.

41. Bakker EN, Bacskai BJ, Arbel-Ornath M, Aldea R, Bedussi B, et al. (2016) Lymphatic Clearance of the Brain: Perivascular, Paravascular and Significance for Neurodegenerative Diseases. Cell Mol Neurobiol 36(2): 181-194.

42. Cserr HF (1971) Physiology of the choroid plexus. Physiol Rev 51(2): 273-311.

43. Nedergaard M (2013) Neuroscience. Garbage truck of the brain. Science 340(6140): 1529-1530.

44. Cserr HF, Cooper DN, Suri PK, Patlak CS (1981) Efflux of radiolabeled polyethylene glycols and albumin from rat brain. Am J Physiol 240(4): F319-328.

45. Hladky SB, Barrand MA (2014) Mechanisms of fluid movement into, through and out of the brain: evaluation of the evidence. Fluids Barriers CNS 11(1): 26.

46. Salameh TS, Banks WA (2014) Delivery of therapeutic peptides and proteins to the CNS. Adv Pharmacol 71: 277-299.

47. Johanson CE, Stopa EG, McMillan PN (2011) The blood-cerebrospinal fluid barrier: structure and functional significance. Methods Mol Biol 686: 101-131.

48. Wang JZ, Xiao N, Zhang YZ, Zhao CX, Guo XH, et al. (2016) Mfsd2a-based pharmacological strategies for drug delivery across the blood-brain barrier. Pharmacol Res 104: 124-131.

49. Morris AW, Sharp MM, Albargothy ,NJ Cheryl A, Ajay Verma, Roy O, et al. (2016) Vascular basement membranes as pathways for the passage of fluid into and out of the brain. Acta Neuropathol 131: 725-736.

50. Eide PK, Ringstad G (2015) MRI with intrathecal MRI gadolinium contrast medium administration: a possible method to assess glymphatic function in human brain. Acta Radiol Open 4(11): 2058460115609635.

51. Iliff JJ, Wang M, Zeppenfeld DM, Venkataraman A, Plog BA, et al. (2013) Cerebral arterial pulsation drives paravascular CSF-interstitial fluid exchange in the murine brain. J Neurosci 33(46): 18190-18199.

52. Ueno M, Chiba Y, Murakami R, Matsumoto K, Kawauchi M, et al. (2016) Blood-brain barrier and blood-cerebrospinal fluid barrier in normal and pathological conditions. Brain Tumor Pathol 33(2): 89-96.

53. Iliff JJ, Wang M, Liao Y, Plogg BA, Peng W, et al. (2012) A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β. Sci Transl Med 4(147): 147ra111.

54. Nakada T (2014) Virchow-Robin space and aquaporin-4: new insights on an old friend. Croat Med J 55(4): 328-336.

55. Iliff JJ, Lee H, Yu M, Feng T, Logan J, et al. (2013) Brain-wide pathway for waste clearance captured by contrast-enhanced MRI. J Clin Invest 123(3): 1299-1309.

56. Solomos AC, Rall GF (2016) Get It through Your Thick Head: Emerging Principles in Neuroimmunology and Neurovirology Redefine Central Nervous System "Immune Privilege". ACS Chem Neurosci 7(4): 435-441.

57. Xie L, Kang H, Xu Q, Chen MJ, Liao Y, et al. (2013) Sleep drives metabolite clearance from the adult brain. Science 342(6156): 373-377.

58. Jessen NA, Munk AS, Lundgaard I, Nedergaard M (2015) The Glymphatic System: A Beginner's Guide. Neurochem Res 40(12): 2583-2599.

59. Lee H, Xie L, Yu M, Kang H, Feng T, et al. (2015) The Effect of Body Posture on Brain Glymphatic Transport. J Neurosci 35(31): 11034-11044.

60. Ball KK, Cruz NF, Mrak RE, Dienel GA (2010) Trafficking of glucose, lactate, and amyloid-beta from the inferior colliculus through perivascular routes. J Cereb Blood Flow Metab 30(1): 162-176.

61. Kress BT, Iliff JJ, Xia M, Wang M, Wei HS, et al. (2014) Impairment of paravascular clearance pathways in the aging brain. Ann Neurol 76(6): 845-861.

62. Yamada K, Cirrito JR, Stewart FR, Jiang H, Finn MB, et al. (2011) In vivo microdialysis reveals age-dependent decrease of brain interstitial fluid tau levels in P301S human tau transgenic mice. J Neurosci 31(37): 13110-13117.

63. Iliff JJ, Chen MJ, Plog BA, Zeppenfeld DM, Soltero M, et al. (2014) Impairment of glymphatic pathway function promotes tau pathology after traumatic brain injury. J Neurosci 34(49): 16180-16193.

64. Engelhardt B, Ransohoff RM (2012) Capture, crawl, cross: the T cell code to breach the blood-brain barriers. Trends Immunol 33(12): 579-589.

65. Brinker T, Stopa E, Morrison J, Klinge P (2014) A new look at

cerebrospinal fluid circulation. Fluids Barriers CNS 11: 10.

66. Louveau A, Harris TH, Kipnis J (2015) Revisiting the Mechanisms of CNS Immune Privilege. Trends Immunol 36(10): 569-577.

67. Louveau A, Smirnov I, Keyes TJ, Eccles JD, Rouhani S, et al. (2015) Structural and functional features of central nervous system lymphatic vessels. Nature 523(7560): 337-341.

68. Aspelund A, Antila S, Proulx ST, Karlsen TV, Karaman S et al. (2015) A dural lymphatic vascular system that drains brain interstitial fluid and macromolecules. J Exp Med 212(7): 991-999.

69. Hitscherich K, Smith K, Cuoco JA, Ruvolo KE, Mancini JD, et al. (2016) The Glymphatic-Lymphatic Continuum: Opportunities for Osteopathic Manipulative Medicine. J Am Osteopath Assoc 116(3): 170-177.

70. McComb JG (1983) Recent research into the nature of cerebrospinal fluid formation and absorption. J Neurosurgeon 59(3): 369-383.

71. Chen L, Elias G, Yostos MP, Stimec B, Fasel J, et al. (2015) Pathways of cerebrospinal fluid outflow: a deeper understanding of resorption. Neuroradiology 57(2): 139-147.

72. Cserr HF, Cooper DN, Milhorat TH (1977) Flow of cerebral interstitial fluid as indicated by the removal of extracellular markers from rat caudate nucleus. Exp Eye Res (25 Suppl): 461-473.

73. GS (1869) Die Arachnoidalraum ein Lymphraum und sein Zusammenhang mit den Perichorioidalraum. Zbl med Wiss Zentralblatt fur die medizinischen Wissenschaften 7: 465-467.

74. Mortensen OA, Sullivan WE (1933) The cerebrospinal fluid and the cervical lymph nodes. The Anatomical Record 56(4): 359-363.

75. Boulton M, Flessner M, Armstrong D, Mohamed R, Hay J, et al. (1999) Contribution of extracranial lymphatics and arachnoid villi to the clearance of a CSF tracer in the rat. Am J Physiol 276(3 Pt 2): R818-R823.

76. Carare RO, Teeling JL, Hawkes CA, Püntener U, Weller RO, et al. (2013) Immune complex formation impairs the elimination of solutes from the brain: implications for immunotherapy in Alzheimer's disease. Acta Neuropathol Commun 1: 48.

77. Plog BA, Dashnaw ML, Hitomi E, Peng W, Liao Y, et al. (2015) Biomarkers of traumatic injury are transported from brain to blood via the glymphatic system. J Neurosci 35(2): 518-526.

78. Groothuis DR, Vavra MW, Schlageter KE, Kang EW, Itskovich AC, et al. (2007) Efflux of drugs and solutes from brain: the interactive roles of diffusion transcapillary transport, bulk flow and capillary transporters. J Cereb Blood Flow Metabolism 27(1): 43-56.

79. Cserr HF, Harling-Berg CJ, Knopf PM (1992) Drainage of brain extracellular fluid into blood and deep cervical lymph and its immunological significance. Brain pathology (Zurich, Switzerland) 2(4): 269-276.

80. Alitalo AK, Proulx ST, Karaman S, et al. (2013) VEGF-C and VEGF-D blockade inhibits inflammatory skin carcinogenesis. Cancer Res 73(14): 4212-4221.

81. Mathieu E, Gupta N, Macdonald RL, Ai J, Yucel YH (2013) *In vivo* imaging of lymphatic drainage of cerebrospinal fluid in mouse. Fluids Barriers CNS 10(1): 35.

82. Girard JP, Moussion C, Forster R (2012) HEVs, lymphatics and homeostatic immune cell trafficking in lymph nodes. Nat Rev Immunol 12(11): 762-773.

83. Laman JD, Weller RO (2013) Drainage of cells and soluble antigen from the CNS to regional lymph nodes. J Neuroimmune Pharmacol 8(4): 840-856.

84. Stern JN, Yaari G, Vander Heiden JA, Church G, Donahue WF, et al. (2014) B cells populating the multiple sclerosis brain mature in the draining cervical lymph nodes. Sci Transl Med 6(248) 248ra107 .

85. EE G (1913) Vitalfarbung am Zentralnerven sytems. Beitrag cur Physio- Pathologie des Plexus choriodeus und der Hirnhaute In: Berlin (Ed.), Austrian Academy of Sciences, Austria.

86. Yoffey JM, Drinker CK (1939) Some observations on the lymphatic's of the nasal mucous membrane in the cat and monkey. J Anat 74(Pt 1): 45-52.

87. McComb JG, Davson H, Hyman S, Weiss MH (1982) Cerebrospinal fluid drainage as influenced by ventricular pressure in the rabbit. Journal of neurosurgery 56(6): 790-797.

88. Brinker T BC, Samii M (1994) A species comparing radiological study on the absorption of cerebrospinal fluid into the cervical lymphatic system. In Intracranial Pressure (IX edn by): Nagai H, Kamiya K and Ishii K. Tokyo, Springer (559-560).

89. Zakharov A, Papaiconomou C, Johnston M (2004) Lymphatic vessels gain access to cerebrospinal fluid through unique association with olfactory nerves. Lymphat Res Biol 2(3): 139-146.

90. Liu H, Ni Z, Chen Y (2012) olfactory route for cerebrospinal fluid drainage into the cervical lymphatic system in a rabbit experimental model. Neural Regen Res 7(10): 766-771.

91. Murtha LA, Yang Q, Parsons MW (2014) Cerebrospinal fluid is drained primarily via the spinal canal and olfactory route in young and aged spontaneously hypertensive rats. Fluids Barriers CNS 6; 11: 12.

92. Ethel DW (2014) Disruption of cerebrospinal fluid flow through the olfactory system may contribute to Alzheimer's disease pathogenesis. J Alzheimers Dis 41(4): 1021-1030.

93. Kinross JM, Drymousis P, Jimenez B, Frilling A (2013) Metabonomic profiling: a novel approach in neuroendocrine neoplasias Surgery. 154(6): 1185-1193; discussion 1185-1183.

94. Mohammad MG, Tsai VW, Ruitenberg MJ (2014) Immune cell trafficking from the brain maintains CNS immune tolerance. J Clin Invest 124(3): 1228-1241.

95. Oreskovic D, Klarica M (2010) The formation of cerebrospinal fluid: nearly a hundred years of interpretations and misinterpretations. Brain Res Rev 64(2): 241-262.

96. Oreskovic D, Klarica M (2011) Development of hydrocephalus and classical hypothesis of cerebrospinal fluid hydrodynamics facts and illusions. Prog Neurobiol 94(3): 238-258.

97. Tokuda T (2014) [Emerging concept of the production and absorption of cerebrospinal fluid and recent progress in the diagnosis and treatment of iNPH]. Rinsho Shinkeigaku 54(12): 1193-1196.

98. Kim H, Moore SA, Johnston MG (2014) Potential for intranasal drug delivery to alter cerebrospinal fluid outflow via the nasal turbinate lymphatics. Fluids Barriers CNS 11(1): 4.

99. Partridge WM (2015) Blood-brain barrier endogenous transporters as therapeutic targets: a new model for small molecule CNS drug discovery. Expert Opin Ther Targets 19(8): 1059-1072.

100. Castagna L, Zanella A, Scaravilli V (2015) affects on membrane lung gas exchange of intermittent high gas flow recruitment maneuver: preliminary data in veno-venous ECMO patients. J Artif Organs 18(3): 213-219.

101. De Giovanni N, Fucci N (2013) The current status of sweat testing for drugs of abuse: a review. Curr Med Chem 20(4): 545-561.

102. Larsen EH, Deaton LE, Onken H (2014) Osmoregulation and excretion. Compr Physiol 4(2): 405-573.

103. Abdel-Mageed SM, Mohamed EI (2016) Comparative modeling of combined transport of water and graded-size molecules across the glomerular capillary wall. J Theor Biol 394: 109-116.

104. Gekle M (2016) Kidney and aging - A narrative review. Experimental gerontology in press. 10.1016/j.exger.2016.03.013.

105. Seppi T, Prajczer S, Dorler (2016) MM Sex Differences in Renal Proximal Tubular Cell Homeostasis. J Am Soc Nephrol.

106. Mischak H, Delles C, Vlahou A, Vanholder R (2015) Proteomic biomarkers in kidney disease: issues in development and implementation. Nat Rev Nephrol 11(4): 221-232.

107. Aregger F, Uehlinger DE, Witowski J (2014) Identification of IGFBP-7 by urinary proteomics as a novel prognostic marker in early acute kidney injury. Kidney Int 85(4): 909-919.

108. Xu XJ, Zheng P, Ren GP (2014) 2,4-Dihydroxypyrimidine is a potential urinary metabolite biomarker for diagnosing bipolar disorder. Molecular bio Systems 10(4): 813-819.

109. Peng J, Guo K, Xia J (2014) Development of isotope labeling liquid chromatography mass spectrometry for mouse urine metabolomics Quantitative metabolomic study of transgenic mice related to Alzheimer's disease. J Proteome Res 13(10): 4457-4469.

110. Ottens AK, Stafflinger JE, Griffin HE, Kunz RD, CifuDX et al. (2014) Post-acute brain injury urinary signature: a new resource for molecular diagnostics. J Neurotrauma 31(8): 782-788.

111. Van Duinkerken E, Ryan CM, Schoonheim MM (2016) Subgenual Cingulate Cortex Functional Connectivity in Relation to Depressive Symptoms and Cognitive Functioning in Type 1 Diabetes Mellitus Patients. Psychosom Med 78(6): 740-749.

112. An M, Gao Y (2015) Urinary Biomarkers of Brain Diseases. Genomics, proteomics & bioinformatics 13(6): 345-354.

113. Raimondo F, Cerra D, Magni F, Pitto M (2016) Urinary proteomics for the study of genetic kidney diseases. Expert Rev Proteomics 13(3): 309-324.

114. Blood Test Results - Normal Ranges. (Ed.^(Eds) (Bloodbook.Com)

115. Deepak A, Rao Le TB, Vikas (2008) First Aid for the USMLE Step 1. McGraw-Hill Medical, USA.

116. (2008) Reference range list from Uppsala University Hospital Laborationslista, Sweden.

117. Gardner MD, Scott R (1980) Age-and sex-related reference ranges for eight plasma constituents derived from randomly selected adults in a Scottish new town. J Clin Pathol 33(4): 380-385.

118. Lu DR, Robinson WH (2014) Street-experienced peripheral B cells traffic to the brain. Sci Transl Med 6(248): 248fs231.