Reduced Serum Cholinesterase Activity Distinguishes Hepatic Encephalopathy From 48 Types of Human Diseases

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Research

Keywords: Serum cholinesterase activity (CHE), Hepatic encephalopathy (HE), Area under curve (AUC), Receiving operator curve (ROC), Accuracy, Sensitivity, and Specificity

Posted Date: December 15th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-126761/v1

Abstract

Background: Hepatic encephalopathy is a complication of central nervous systems due to liver failure-related brain inflammation. Less than half of patients suffering from liver failure develop hepatic encephalopathy, which suggests other factors beyond liver failure might contribute to hepatic encephalopathy. Indeed, we reported previously that the levels of serum direct bilirubin, a liver cell-made product, are counter-intuitively highest in hepatic encephalopathy patients among 72 clinically defined diseases. In current study, we tested if cholinesterase could serve as a biomarker for hepatic encephalopathy by comparing serum cholinesterase activities among 48 different types of human diseases.

Methods: The activity of serum cholinesterase was determined by the standard “continuous monitoring method” in the clinical laboratory of the hospital where the serum cholinesterase activities from 137,305 independent tests with 48 clinically defined diseases and 3,387 independent tests from healthy individuals who came to the hospital for physical examination during the past 5 years were retrieved. All data were analyzed with RStudio V.1.3.1073 and python libraries 3.8.

Results: We found that all 48 types of diseases had decreased cholinesterase activity compared to control based on either mean or median values. Remarkably, hepatic encephalopathy had the lowest cholinesterase activity and the serum cholinesterase activity was the best biomarker for hepatic encephalopathy (AUC 0.99, sensitivity 100%, and specificity 99%) among all diseases. Moreover, two component analysis of cholinesterase activity distributions revealed hepatic encephalopathy resembled preeclampsia and uremia whereas cirrhosis resembled multiple myeloma, leukemia, myeloproliferative disorder, and liver cancer.

Conclusions: Decreased cholinesterase activity was an almost perfect serum biomarker for patients suffering hepatic encephalopathy at all stages. The resemblance of hepatic encephalopathy to preeclampsia and uremia based on cholinesterase activities provided new insight in understanding hepatic encephalopathy etiology beyond liver failure.

Background

Hepatic encephalopathy (HE) is known as a spectrum of neuropsychiatric abnormalities in patients suffering from liver dysfunction when other known brain disease has been excluded[1, 2]. HE is not only associated with systemic inflammation, but also with neuroinflammation [3–5] or endotoxemia[6, 7]. Its onset may be gradual or sudden manifested as neuropsychiatric disorders and motor dysfunctions[8]. The one-year mortality of severe HE is 54%[9]. HE is divided into covert type including minimal HE and grade I HE, and overt type ranging from grade II to grade IV HE[10]. Increased serum ammonia[11], γ-aminobutyric acid[12], manganese[13], or oxidative/nitrosative stress, etc. are correlated to the occurrence and development of HE[14]. Lactone, rifaximin, probiotics or molecular adsorbent recirculating system that have anti-inflammatory effects can significantly improve the neuropsychiatric symptoms of patients.

Even though HE is associated with liver failure, less than half of patients suffering liver failure develop HE, suggesting other factors contributed to HE development. Indeed, we found that the levels of serum direct bilirubin, a liver cell-made product, are counter-intuitively highest in HE patients among 72 clinically defined diseases[15]. In order to monitor therapeutic effects for patients suffering from HE, blood-based biomarkers are desirable. High levels of blood ammonia are usually used to monitor the therapeutic effects of HE. However, recent studies have shown that concentrations of blood ammonia cannot always reflect the severity of HE [16–18]. Therefore, it is necessary to explore other serum biomarkers with better sensitivity and specificity for monitoring the therapeutic effects of HE and revealing more etiology of HE.

Serum CHE is a serine hydrolase mainly synthesized in liver, which is also expressed in pancreas, glia, and the endothelial cells of the central nervous system. CHE consists of four identical subunits with 574 amino acids each and each monomer contains ten potential N-glycosylation sequences in which nine are glycosylated[19, 20]. CHE is centered on a proline-rich polypeptide and the four identical subunits interweave at the C-terminal to form the mature tetramer[21]. It comes to realize that CHE plays a vital role in the hydrolysis of different esters, including butyrylcholine, succinylcholine and ghrelin[22], and is also involved in the metabolism of certain drugs and toxic substance, such as procaine, cocaine, heroin[23, 24], aspirin, and organophosphorus poisons[25, 26]. In addition, CHE is associated with inflammation and parasympathetic dysfunction[27, 28]. However, the serum CHE activities and their dynamic ranges have never been studied and compared systematically in different types of diseases.

In current study, the serum CHE levels from 137,305 patients with 48 clinically defined diseases and 3,387 healthy individuals during the same period were retrieved from the clinical laboratory of the Affiliated Hospital of Qingdao University during the past 5 years and different statistical methods were employed to perform data analysis.

Methods

Study participants

After obtaining approval from the Hospital Ethics Review Board of Qingdao University, we were allowed to retrieve the electronic medical records and lab data of serum CHE activities of both healthy individuals and patients with clinically defined diseases from the clinical laboratory of the Affiliated Hospital of Qingdao University during the past 5 years. All research was performed in accordance with relevant guidelines/regulations and informed consent was obtained from all participants and/or their legal guardians.

Clinical Data Collection

All diseases with more than 84 independent serum CHE activity tests over past 5 years were chosen. All patients with primary diagnosis of specific disease were included in current study without exclusions. Thus, all diseases include all patients at different stages of disease development with or without medical

interventions. However, we excluded the 2.5% highest and the 2.5% lowest CHE activities for each disease. As results, 137,305 CHE activities from patients suffering from 48 types of diseases and 3,387 CHE activities from healthy controls were used for data analysis.

Measurements Of Serum Che Activities

Various methods have been used to determine the serum CHE activity during the past[29], but the use is limited due to their limitations. Classical spectrophotometric Ellman's method is used widely to determine activity of CHE due to its advantages of being fast, simple, cheap and accurate. The method involves two steps. Firstly, butylthiocholine is converted into butyric acid and thiocholine under the action of serum CHE. Thiocholine is then reacted with 5,5'-dithiobis-(2-nitrobenzoic) spontaneously, which produces 5-thio-2-nitrobenzoic acid and 2-nitrobenzoic acid-5-thiocholine[30, 31]. The activity of CHE in the sample is calculated by measuring the increased absorbance of 5-thio-2-nitrobenzoic acid at 410 nm. The serum CHE assay used by the clinical lab in our hospital is "the continuous monitoring method" with the same principle of the spectrometric Ellman's method.

ROC analysis. ROC curves were plotted using SPSS v26 (IBM, Armonk, US). Youden's indices were calculated using ROC curve coordinates in order to determine AUCs, accuracy, sensitivity and specificity at the point where test performance is optimal.

Statistical analysis

All data retrieved was analyzed with RStudio V.1.3.1073 (RStudio, Boston, USA) and python libraries 3.8 (Anaconda Software Distribution). The results were demonstrated as median values and means ± standard deviation (SD). Standard t-test was used to compare the clinical characteristics of subjects in the specific disease and control groups. Median levels of serum CHE activities between groups were compared by means of the Mann-Whitney U-test. Groups were compared using the Kruskal-Wallis test (a non-parametric one-way ANOVA). Logistic regression was used to test the interactive effects of other variables on the observed association. $P < 0.05$ was considered as being statistically significant.

Results

Based on the lab data of serum CHE activities retrieved from the clinical lab of our hospital over the past 5 years, the number of cases, mean (standard deviation,SD), median (interquartile ranges) and P values for each of the 48 diseases in comparison to healthy controls were summarized in Table 1.

Table 1
Serum CHE activities (U/L) in 48 clinically defined diseases in patients and in healthy controls

CHE	# of cases	Mean (SD)	Median (IQR)	*p* Value
Hepatic Encephalopathy	82	3640.8 (1221.9)	3633.5 (2757.8, 4672.0)	< 0.001
Cirrhosis	8,073	4702.4 (2321.8)	4032.0 (2853.0, 6290.0)	< 0.001
Sepsis	91	4830.6 (2007.4)	4555.0 (3253.0, 6366.5)	< 0.001
Pancreatic Cancer	944	5542.7 (2036.8)	5459.0 (4047.0, 7144.5)	< 0.001
Liver Cancer	286	6032.7 (2163.7)	5939.5 (4325.3, 7680.5)	< 0.001
Myeloproliferative Disorder	1,022	6113.4 (2137.3)	6069.0 (4555.5, 7743.3)	< 0.001
Chronic Obstructive PD	1,298	6357.7 (1966.6)	6321.5 (4910.3, 7853.3)	< 0.001
Gastric Cancer	10,414	6386.1 (1772.5)	6322.5 (5098.3, 7595.0)	< 0.001
Brain Trauma	538	6492.5 (1953.3)	6405.0 (5009.8, 7872.0)	< 0.001
Uremia	5,703	6388.7 (1497.7)	6435.0 (5306.5, 7437.0)	< 0.001
Esophagus Cancer	3,060	6505.7 (1748.8)	6498.5 (5181.8, 7827.3)	< 0.001
Pancreatitis	1,376	6610.6 (2009.6)	6555.5 (5207.8, 7999.5)	< 0.001
Anemia	1,792	6714.8 (2064.1)	6555.0 (5201.0, 8196.8)	< 0.001
Intracranial Hemorrhage	3,075	6833.2 (1911.4)	6754.0 (5330.0, 8239.0)	< 0.001
Preeclampsia	728	6789.1 (1469.1)	6809.5 (5766.5, 7819.3)	< 0.001
Azotemia	357	6997.0 (2067.9)	6921.0 (5341.0, 8598.0)	< 0.001
Encephalitis	449	7044.4 (1949.1)	7023.0 (5557.0, 8515.0)	< 0.001
Lung Fibrosis	244	7040.6 (2013.8)	7049.0 (5581.8, 8555.3)	< 0.001
Leukemia	4,228	7085.5 (2247.5)	7119.5 (5387.8, 8793.0)	< 0.001
Colon Cancer	5,279	7255.5 (1974.5)	7267.0 (5796.5, 8717.0)	< 0.001
Acute Myocardial Infarction	1,877	7354.7 (1806.1)	7378.0 (6059.0, 8637.0)	< 0.001
Rectum Cancer	6,663	7411.6 (1897.1)	7408.0 (6022.5, 8765.0)	< 0.001
Aplastic Anemia	816	7486.3 (2030.8)	7502.5 (5932.5, 8931.8)	< 0.001
Multiple Myeloma	1,810	7440.5 (2291.6)	7710.5 (5576.5, 9279.8)	< 0.001

Healthy Control is bolded to make easy comparison. SD: standard deviation, IQR: interquartile range. Chronic Obstructive PD: Chronic Obstructive Pulmonary Disease.

CHE	# of cases	Mean (SD)	Median (IQR)	*p* Value
Bone Fracture	1,335	7693.0 (1988.4)	7785.0 (6330.5, 9198.0)	< 0.001
Acute Cerebral Infarction	7,108	7814.3 (1815.8)	7868.0 (6594.0, 9132.3)	< 0.001
Lymphoma	3,543	7789.0 (2409.0)	8037.0 (6142.0, 9559.5)	< 0.001
Coronary Heart Disease	16,725	8023.3 (1955.6)	8190.0 (6679.0, 9494.0)	< 0.001
Rheumatoid Arthritis	455	8190.0 (1750.7)	8278.0 (7005.5, 9428.3)	< 0.001
Diabetic Nephropathy	484	8131.9 (2519.0)	8300.0 (6165.3, 10103.3)	< 0.001
Asthma	462	8183.7 (1731.1)	8326.5 (7071.5, 9472.8)	< 0.001
Lupus Erythematosus	1,218	8374.4 (2004.0)	8463.5 (6883.0, 9910.5)	< 0.001
Hepatitis	6,600	8204.9 (2440.8)	8478.5 (6836.0, 9834.0)	< 0.001
Cerebral Ischemia	1,698	8434.6 (1690.6)	8502.5 (7293.8, 9646.5)	< 0.001
Lung Cancer	8,725	8477.7 (1833.1)	8577.0 (7250.0, 9798.0)	< 0.001
Nephritis	1,780	8692.2 (2181.4)	8689.5 (7260.8, 10229.3)	< 0.001
Cerebral Arteriosclerosis	533	8551.5 (1905.0)	8755.0 (7364.0, 9888.0)	< 0.001
Gastritis	2,630	8813.8 (1676.1)	8819.5 (7666.3, 10012.3)	< 0.001
Cervical Cancer	1,800	9033.0 (1638.2)	9003.5 (7869.5, 10195.0)	< 0.001
Kidney Cancer	1,295	9002.3 (1752.8)	9055.0 (7894.5, 10116.0)	< 0.001
Type 2 Diabetes Mellitus	8,803	9018.4 (1840.8)	9086.0 (7805.5, 10341.5)	< 0.001
Ovarian Cancer	1,966	8955.8 (1989.5)	9133.5 (7686.8, 10398.8)	< 0.001
Gout	875	9055.4 (1931.6)	9228.0 (7727.0, 10483.0)	< 0.001
Ankylosing Spondylitis	95	9033.3 (2122.7)	9417.0 (7512.0, 10666.5)	< 0.001
Breast Cancer	4584	9396.0 (1566.0)	9435.5(8292.5, 10505.0)	< 0.001
Endometrial Cancer	972	9346.5 (1669.2)	9460.5 (8220.8, 10599.0)	< 0.001
Psoriasis	130	9281.7 (1828.4)	9541.5 (7748.5, 10630.0)	< 0.001
Nephrotic Syndrome	3284	9807.8 (2455.5)	9639.5(8000.5, 11440.3)	< 0.001

Healthy Control is bolded to make easy comparison. SD: standard deviation, IQR: interquartile range. Chronic Obstructive PD: Chronic Obstructive Pulmonary Disease.

CHE	# of cases	Mean (SD)	Median (IQR)	*p* Value
Healthy Control	**3,387**	**10055.3 (1548.4)**	**10051.0 (8919.0, 11159.5)**	**-**

Healthy Control is bolded to make easy comparison. SD: standard deviation, IQR: interquartile range. Chronic Obstructive PD: Chronic Obstructive Pulmonary Disease.

All 48 different types of diseases had reduced levels of serum CHE activities in comparison to that in healthy controls with statistical significance ($p<0.001$,Table 1). Among the 48 diseases, patients diagnosed with HE had the lowest median levels of CHE activity, which were followed by cirrhosis, sepsis, pancreatic cancer and liver cancer (Table 1).

To evaluate the diagnostic properties of serum CHE activities as serum biomarkers, the receiving operator curve (ROC) analysis was performed for 48 different types of diseases (Supplemental Fig. 1). Based on the ROC analysis, the area under the curve (AUC), accuracy, sensitivity and specificity for all diseases were listed in Fig. 1. Among the 48 types of diseases studied, 39 of them had the AUCs over 0.60 ranging from 0.60 to 1.00. Remarkably, the serum CHE activity was the best biomarker for HE among all 48 diseases with the AUC of 1.00, sensitivity 100%, and specificity 99%, which were followed by sepsis (AUC = 0.97), liver cancer (AUC = 0.92), myeloproliferative disorder (AUC = 0.92), and pancreatitis (AUC = 0.88). Interestingly, the serum CHE activities had the lowest AUCs for breast cancer and nephrotic syndrome, being 0.51 and 0.52, respectively.

We further noticed that all kidney-related diseases including diabetic nephropathy, gout, kidney cancer, nephritis, lupus erythematous, and nephrotic syndrome and all female-related cancers including cervical, ovarian, and breast cancers had relatively low sensitivities (0.14–0.43) and high specificities (0.79–0.95) when CHE activities were calculated as serum biomarkers (Fig. 1).

To comprehend the observations, we divided the 48 diseases into 6 major categories as cancers, autoimmune diseases, cardio- and cerebrovascular diseases, blood-related diseases (including blood cancers), kidney diseases, and others with different color-code. To visualize the results, we made boxed plots of serum CHE with lower quartile (25%), median (50%), and upper quartile (75%) ranges, and 95% confident intervals marked for 48 clinically defined human diseases and healthy controls and shown in Fig. 2.

HE had not only the lowest median level (50%) but also the lowest 2.5%, 25%, 75%, and 95% of serum CHE activities among all diseases. In contrast, nephrotic syndrome was the only disease that had 75% of serum CHE activities higher than that of control. In addition, diabetic nephropathy, hepatitis, and nephritis were the three diseases that had 97.5% of serum CHE activities higher than that of control. These observations led to speculate that the extreme serum CHE activities were the characteristics of specific diseases. We then assumed that further studying such a relationship might reveal their relationship with HE and cirrhosis.

Thus, we analyzed the statistical features of serum CHE activities in six classes of diseases against HE and cirrhosis according to mean values, standard deviation, 2.5, 25, 50, 75, and 97.5 percentiles. The results of two component analysis of all 48 diseases were shown in Fig. 3.

The apparent clustering of the same category of diseases, such as cancers (at upper side of chart), autoimmune disease (at left side of chart), blood-related diseases (at low right of chart) based on the statistical analysis. Except for HE, diseases directly related to the kidney, such as nephritis, diabetic nephropathy and nephrotic syndrome, were clustered at the low left side of chart. Most impotantly, the two component analysis revealed HE resembled preeclampsia and uremia whereas cirrhosis resembled multiple myeloma, leukemia, myeloproliferative disorder, and liver cancer as shown in Fig. 3.

Discussion

All 48 different types of diseases studied in the current study had the lower median serum CHE activities than that of healthy controls (Table 1), implicating that decreased serum CHE might be a common feature of human diseases. Most of publications reported the dynamic changes of serum CHE activities in one specific disease[32–40], we performed the systematical comparison of serum CHE activities (Table 1) and CHE as biomarkers (Fig. 1) in 48 different types of diseases for the first time. Moreover, serum CHE had almost perfect AUC, accuracy, sensitivity and specificity for HE diagnosis (Fig. 2). Meanwhile, serum CHE activities were also decent serum biomarkers for liver cirrhosis, sepsis and pancreatitis (Fig. 2). Furthermore, the two component analysis results shown in supplemental Fig. 3 and Fig. 3 revealed the resemblance of HE to preeclampsia and uremia were more than that to cirrhosis, which provided new insight in understanding HE etiology beyond liver failure. Thus, understanding the molecular mechanism behind the data presented in current study may be helpful in discovering new molecular targets for HE prevention and treatment.

Increased blood ammonia levels have been considered as the main pathophysiological mechanism of HE[41, 42] by causing swelling astrocytes, activation of microglia and neuroinflammation [43–50]. Microglia activation can promote the proliferation and release of pro-inflammatory cytokines[50], and the inflammation state can eventually lead to neuronal death[51]. In addition, astrocytes cultivated with pro-inflammatory cytokines and interferon gamma (INF-γ) becomes edematous,which indicates the potential function of pro-inflammatory cytokines in the process[52, 53]. Several studies have shown that severe systemic inflammation can induce [54–56] and aggravate HE in cirrhosis[57]. Coltart et al [58] demonstrated that sepsis can induce HE by affecting blood ammonia metabolism and promoting the release of pro-inflammatory mediators. The current study showed that the resenblance of HE to sepsis (Fig. 3), which is consistent with their conclusion.

Inflammation plays a vital role in neurodegenerative diseases[51, 59], such as Alzheimer's disease[60], dementia with Lewy bodies[61], Wilson’s disease[62], Parkinson's disease[63], multiple sclerosis[64]. Serum CHE activities is closely related to the inflammation and is involved in many pathophysiological processes of nervous system diseases[65]. Darreh-Shori et al[66] have shown that serum CHE plays an

important role in regulating intrathecal cytokine and activity of cholinoceptive glial cells in Alzheimer's disease. Macdonald et al [67] showed that CHE has good sensitivity and specificity in the diagnosis of Alzheimer's disease. However, the retrospective CHE activities retrieved in our current study did not provide enough independent test results for other neurodegenerative diseases. Further prospective studies should be useful in addresing these issues.

Chronic hepatitis, especially chronic hepatitis B, is the most common cause of cirrhosis and hepatic cancer in China. Hepatic diseases usually progress from hepatitis to cirrhosis and hepatic cancer. Previous studies have shown that CHE levels of hepatitis and cirrhosis are lower than that in healthy controls, which is consistent with the result of the study. Our data showed that serum CHE levels in HE was the lowest not only in liver-related diseases [68]but also in all other diseases included in current study.

Conclusions

The current study shows that serum CHE was an almost perfect serum biomarker for patients suffering HE at all stages. The resemblance of HE to preeclampsia and uremia based on CHE activities provided new insight in understanding HE beyond liver failure. The etiology of HE deserves further exploration.

Abbreviations

CHE:Serum cholinesterase activity; HE:Hepatic encephalopathy; AUC:Area Under the Curve; ROC:Receiving Operator Curve; SD:Standard Deviation; IQR: Interquartile Range; Chronic Obstructive PD: Chronic Obstructive Pulmonary Disease; NF-γ:Interferon gamma

Declarations

Ethics approval and consent to participate

The Hospital Ethics Review Board of Qingdao University approved current study. All research was performed in accordance with relevant guidelines/regulations and informed consent was obtained from all participants and/or their legal guardians.

Consent for publication

All authors read and approved the manuscript and the final submission.

Availability of data and materials. All data files are available upon request. Correspondence and requests for the data files should be addressed to H.P and L.Z.

Data analysis. Correspondence and requests for detailed data analysis should be addressed to H.P, Y.G., and L.Z.

Competing interests

All authors declare that they have no competing interests.

Funding

This research was supported by the Natural Science Foundation of China (Grants 81672585 and 11804151), and the Taishan Scholar Fellowship to L.Z.

Authors' contributions Y. R., M.Z., Y.Z., Y.Z., L.Z., Y.G., and H.P had full access to all data in the study and takes responsibility for integrity of the data and accuracy of the data analysis. Concept and design: Y.R., M.Z., L.Z., Y.G., and H.P ; acquisition, analysis or interpretation of data: Y. R., M.Z., Y.Z., Y.Z., L.Z., Y.G., and H.P; statistical analysis: Y.R., M.Z., Y.Z., Y.G., and L.Z.; obtaining funding: L.Z. and Y.G.

Ackowledgements We thank all the stuff and patients in our hospital who made current study possible. We are also grateful to all members of the Systems Biology & Medicine Center for Complex Diseases who have helped the project in various ways.

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Figures

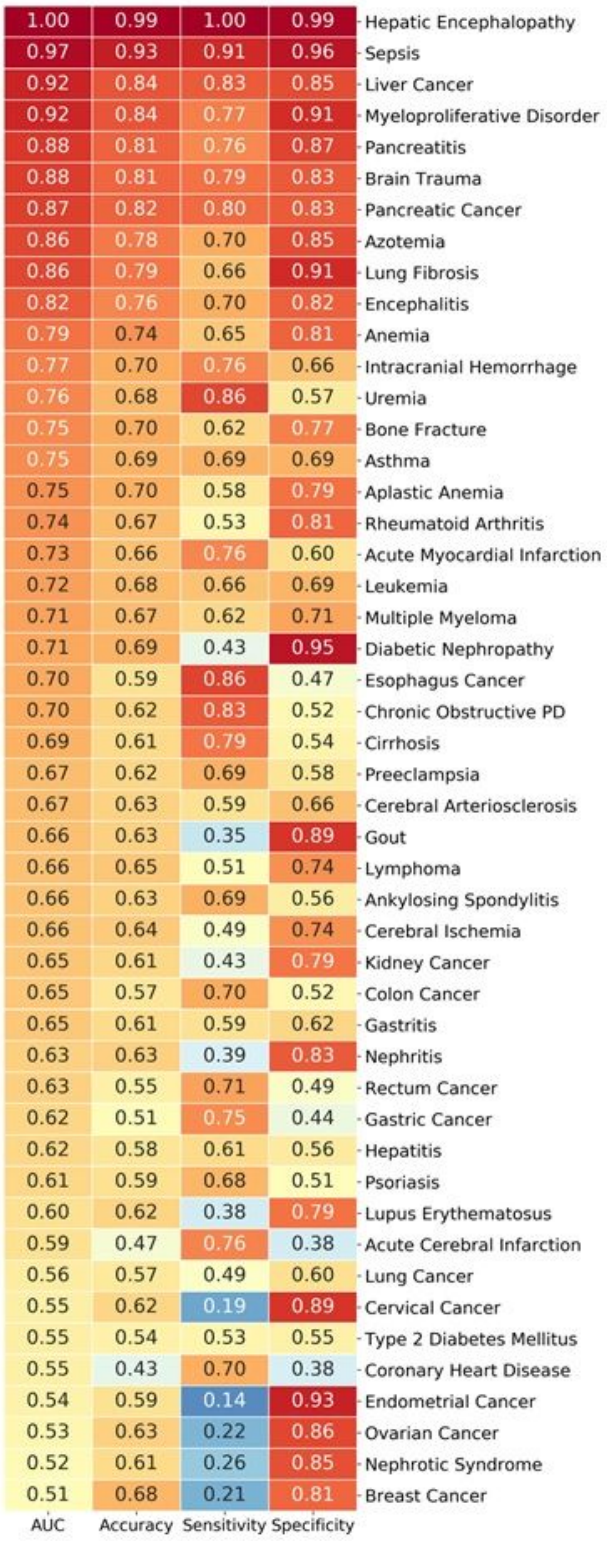
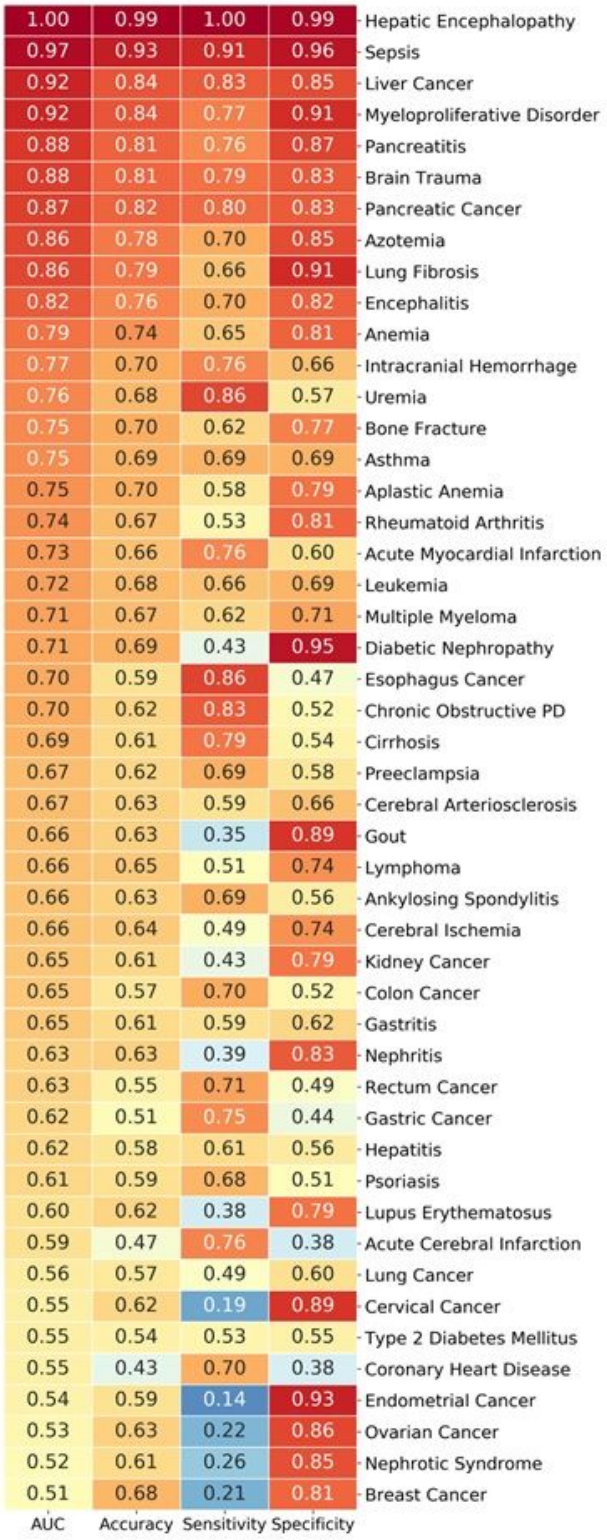


Figure 1

The AUC, accuracy, sensitivity and specificity of serum CHE activities for 48 types of human diseases. AUC: Area under the curve. Chronic Obstructive PD: Chronic Obstructive Pulmonary Disease.



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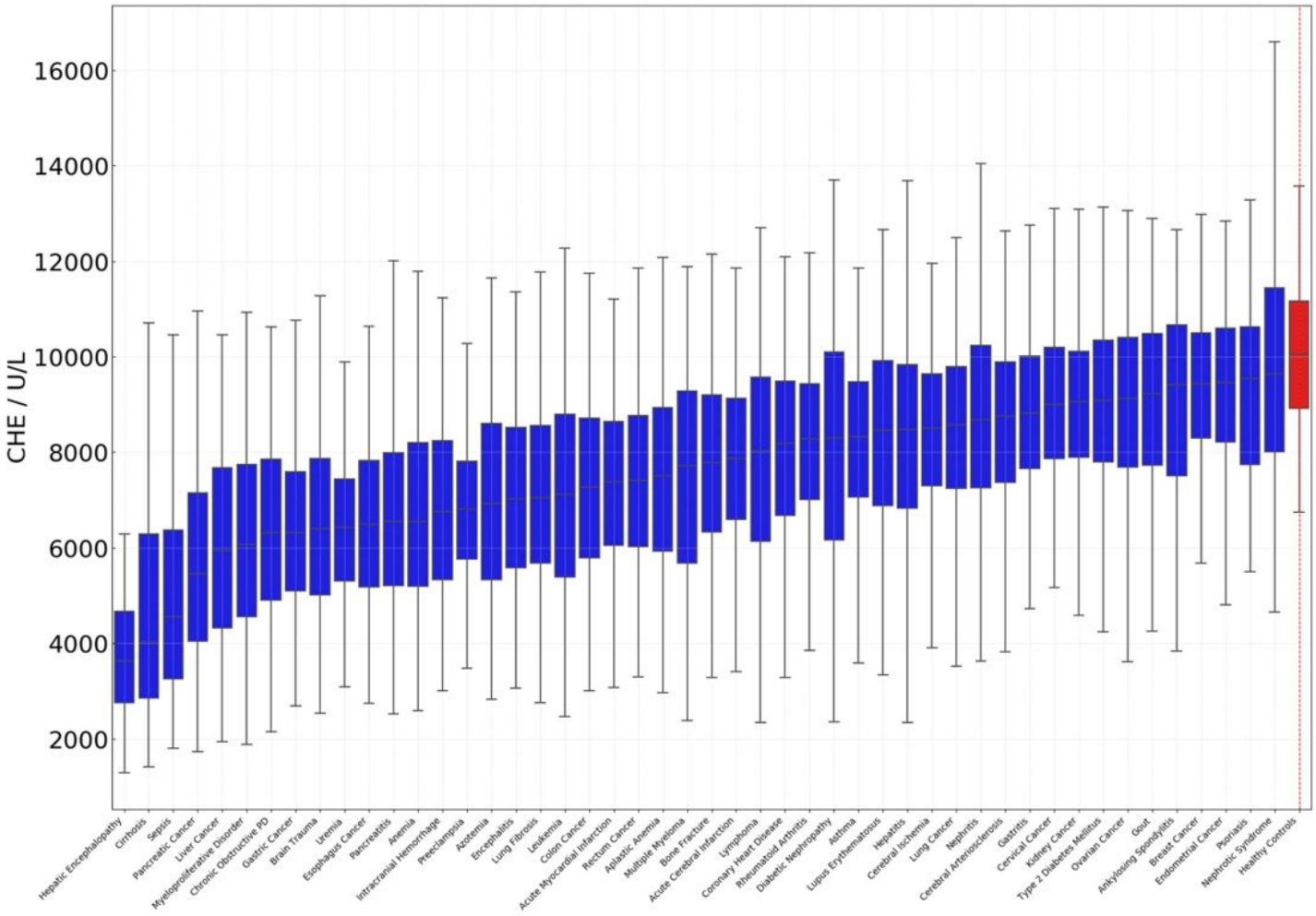
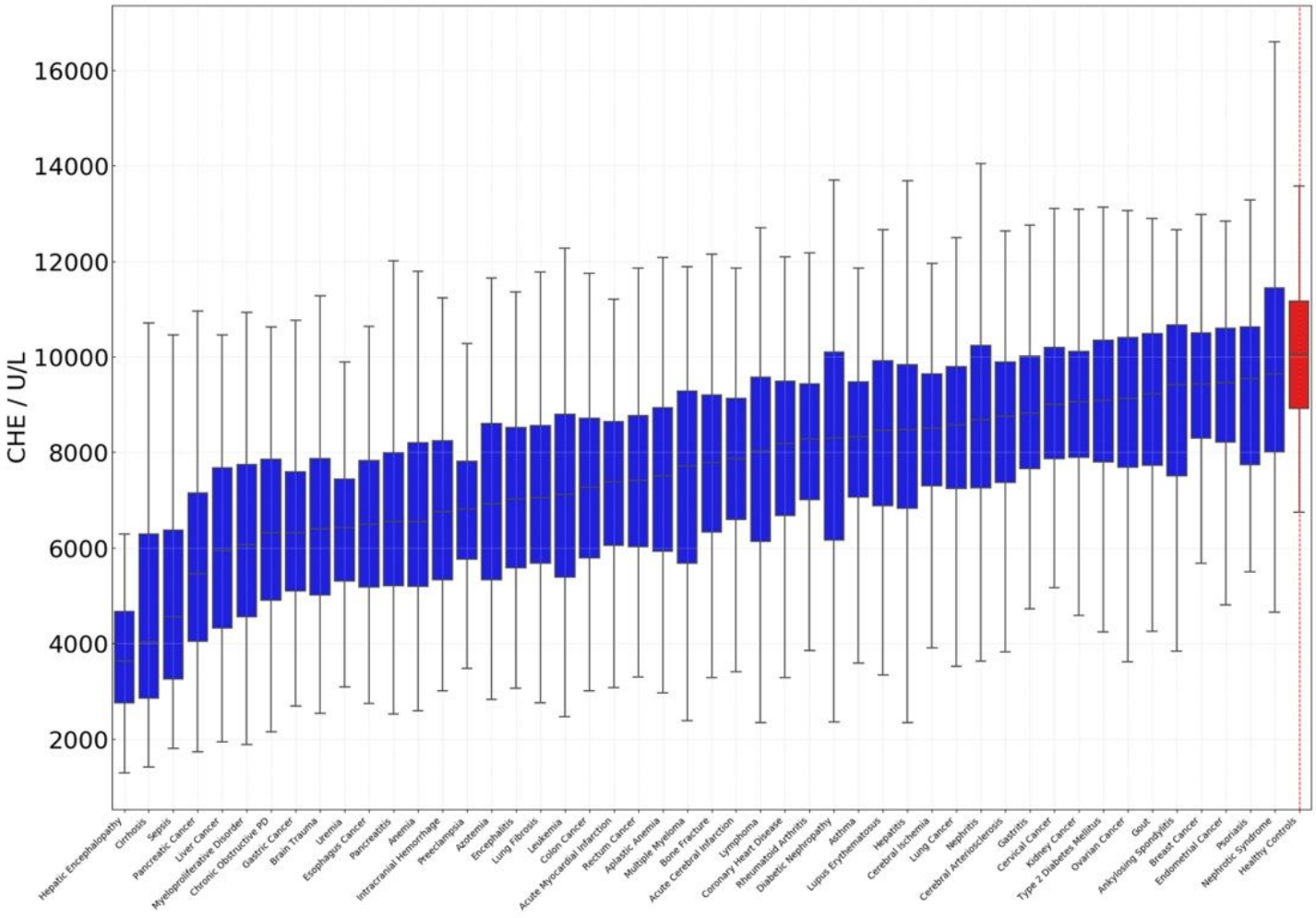


Figure 2

Serum CHE activities in 48 different types of diseases and healthy controls. The data of serum CHE was arranged in an ascending order according to the median values. Chronic Obstructive PD: Chronic Obstructive Pulmonary Disease.



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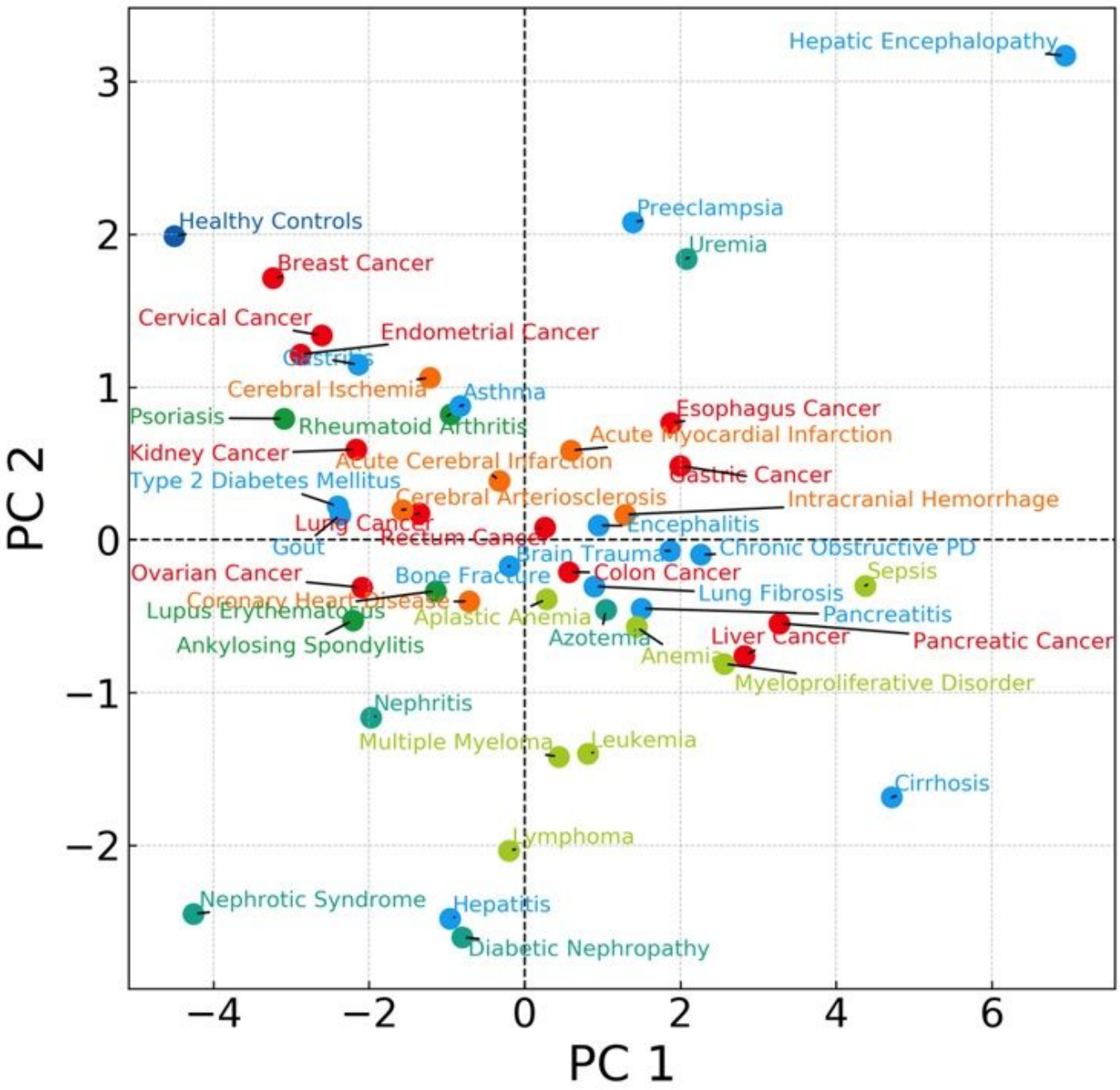
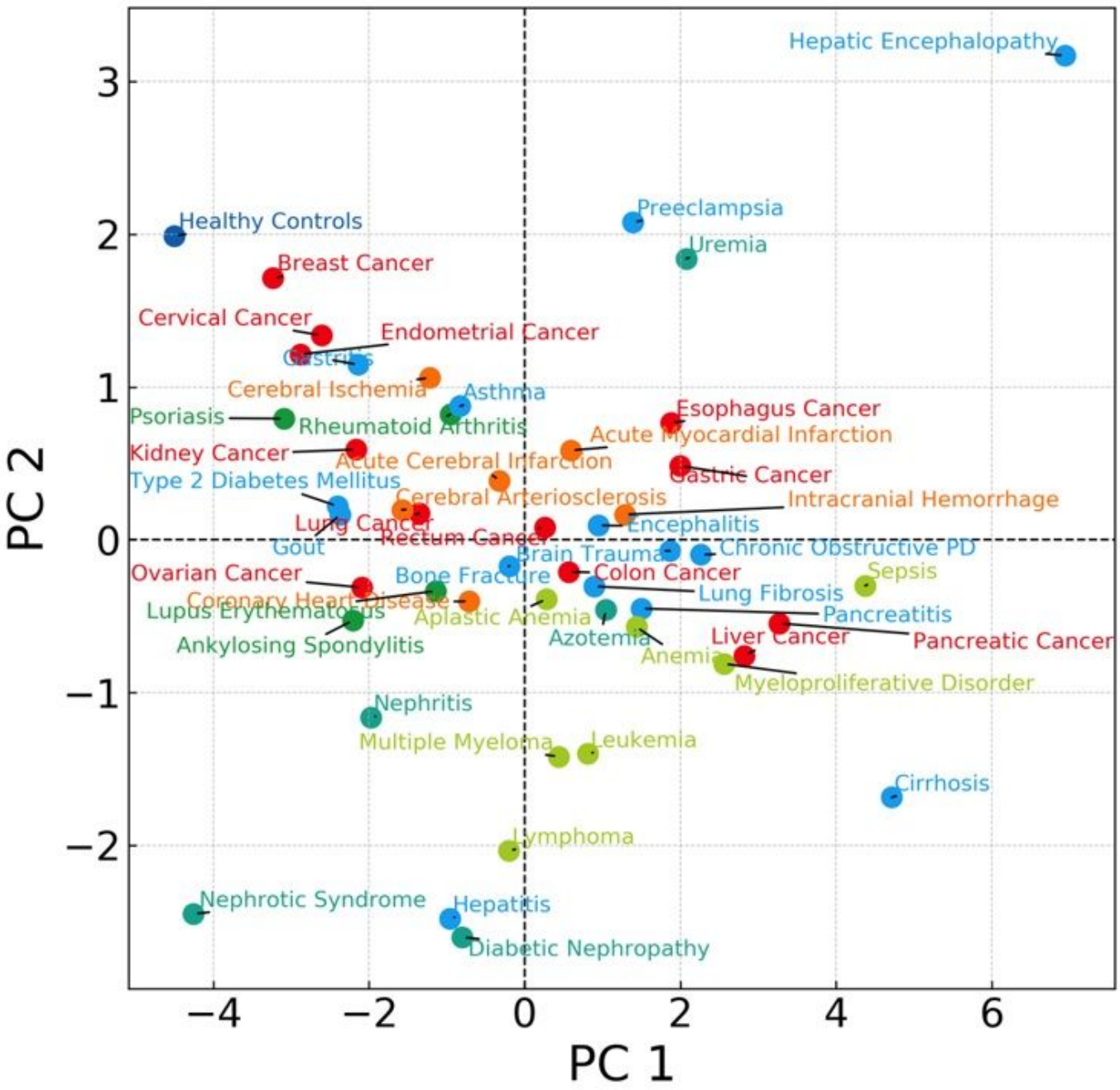


Figure 3

The serum CHE activities had common features in the same class of diseases. The 48 diseases were grouped into 6 major categories including cancers, autoimmune diseases, cardiovascular and cerebrovascular diseases, blood-related diseases, kidney diseases, and others, being marked with red, green, orange, yellow green, teal, and blue, respectively. The statistical features of serum CHE activities of 48 diseases including the mean values, standard deviation, min/max values, 25, 50, and 75 percentiles were quantified. The obtained statistical features of all diseases were further decomposed into two components and presented.



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