Circulating Transthyretin is a Systems Biomarker: Retrospective Clinical Evaluations in 48 Types of Human Diseases

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Research

Keywords: Transthyretin (TTR), Receiving operator curve (ROC), Area under the Curve (AUC), Systems biomarker, Principal component analysis, Heatmap

Posted Date: June 11th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-567632/v1

Abstract

Background: Most human diseases are systems diseases, and systems biomarkers will be powerful tools in facilitating the diagnosis, prognosis, and treatment effect monitoring of systems diseases. Circulating transthyretin (TTR) or prealbumin, one of the commonly ordered blood tests, has been indicated as a biomarker for cancers and non-cancer diseases. To test if TTR were a systems biomarker and the mechanisms of its regulation, TTR concentrations in 48 different types of human diseases and healthy controls were systematically analyzed in the current study.

Methods: Serum TTR concentration was determined by the standard "Immune turbidimetric method" in the clinical laboratory of our hospital. TTR data from 137,305 independent tests of patients with 48 clinically defined diseases and 3,387 tests from healthy individuals who came to the hospital for physical examination during the past five years were retrieved. All data were statistically analyzed with SPSS v26, RStudio V.1.3.1073, and python libraries 3.8.

Results: The mean and median values of serum TTR concentrations were significantly decreased ($p<0.001$) in 47 out of 48 diseases. However, the dynamic TTR concentration ranges were far exceeded that in healthy controls, including patients suffering uremia, the only disease that had significantly increased ($p<0.001$) TTR mean and median values. ROC analyses showed that TTR was a decent biomarker (AUC > 0.6) for 40 out of 48 diseases, and the highest AUCs were observed in sepsis (AUC 0.97), cirrhosis (AUC 0.97), and pancreatitis (AUC 0.94). Remarkably, the eight diseases with AUC < 0.6 were mainly kidney- and blood-related diseases with unconventional ROC curves and broad TTR concentration ranges. Furthermore, similar TTR concentration distributing patterns were present in the same category of diseases, such as different types of cancers, liver-related diseases, blood-related cancers and diseases, vascular diseases, autoimmune diseases, and kidney diseases.

Conclusions: Significantly decreased TTR mean and median values were common in 47 out of 48 human diseases. The liver, kidney, blood, and pancreas were the major organs involved in regulating TTR concentrations. Systems swings between catabolic and anabolic phases explained the different dynamic TTR concentration ranges in different diseases. Overall facts plus ROC analysis indicated that TTR was a systems biomarker instead of a prognosis or malnutrition indicator used clinically during the past.

Background

Most aging-dependent human diseases are associated with multiple problems, and patients usually take several prescription medicines. Employing systems biology, bioinformatics, and OMICS approaches, systems biomarkers have been pursued in different types of human disease to judge the overall treatment effects and monitor possible side effects.[1–3] However, none has been translated into clinical use due to technical difficulties. Quantitative and straightforward blood test-based assays are preferred for systems biomarker development.

To accomplish such a goal, our laboratory has taken two independent approaches. We have discovered that the blood glucose to mannose ratio, containing glucose-dependent energy status and glucose- and mannose-related glycosylation homeostasis information, serves as an excellent systems biomarker for 39 types of human diseases[4]. We have also re-investigated 17 glucose and glycan-related clinical blood tests, including ten cancer biomarkers as systems biomarkers[5, 6]. Based on the mean plus median levels, p values, and dynamic ranges of the 17 clinical blood tests from 1.4 million clinical samples in 64 human diseases[7], our published data showed that most of the abnormal lab results, including increased serum cancer biomarker levels, are indicators of systems malfunction [5, 8]. Based on these results, we continued our effort in searching systems biomarker candidates from clinical blood tests.

Circulating transthyretin (TTR) is a sound systems biomarker candidate. TTR has been reported as biomarkers for both cancers and non-cancerous chronic and acute diseases[9–19], indicating tumors, inflammation, trauma, autoimmune, and other disorders are associated with decreased circulating TTR levels. Moreover, TTR was proposed and used as an indicator of protein nutritional status in the Lancet since 1972[20]. Subsequent evidence does not support the use of aggressive nutritional support to attempt normalization of serum TTR levels in patients[9]. Overtreatment of presumed protein-energy malnutrition is probably not detrimental to most patients, and the failure to detect other natural causes of decreased TTR concentrations might be disastrous.

The liver secretes transthyretin directly into the blood. In contrast, the choroid plexus secretes TTR into the cerebrospinal fluid, then gets into the blood circulation. Since the liver is the major source of TTR in blood circulation, TTR concentrations are significantly reduced in patients who have cirrhosis, hepatitis, and obstructive jaundice[21]. However, it is largely unknown why circulating TTR concentrations are dramatically reduced in liver-unrelated diseases. For example, reduced TTR concentration is a predictor of prognosis in patients with acute leukemia, colorectal, pancreatic, breast, esophageal, ovarian, lung, and gastric cancers [16, 22–30]. TTR is also closely related to heart failure [31, 32], postoperative complications[33], acute coronary syndrome (ACS) [13–15], inflammatory process[34], wound healing[35], and renal injury[9, 36, 37]. TTR alone can predict morbidity and mortality in a variety of surgical and non-surgical patients, such as hemodialysis[38], stomach surgery[34], heart surgery[33], hepatectomy[39], pneumonectomy complications[40], burn patients[10] stroke[19, 41, 42], and COVID-19 infections[43, 44]. Thus, abnormal circulating TTR concentration is a general disease indicator regulated at the systems level.

TTR was originally named prealbumin for it runs before albumin on electrophoresis gels. TTR transports thyroid hormone thyroxine and retinol-binding protein-bound to retinol in blood circulation [45]. It is encoded by the TTR gene located in chromosome 18 q12.1. Besides its transporter properties, TTR has thymic hormone activity that can enhance the body's immunity by promoting the maturation of lymphocytes[46]. TTR also binds to βamyloid protein and prevents its natural tendency to aggregate, which is thought to help prevent amyloid plaque formation and even treat Alzheimer's disease [47]. The hormone transporting and other functions of TTR explain its general biomarker properties in different types of human diseases. However, the dynamic changes in circulating TTR concentrations in different

types of human diseases have never been studied and compared systematically to reveal the general mechanisms of its regulation.

In the current study, the TTR concentrations from 137,305 independent tests of patients suffering 48 clinically defined diseases and 3,387 tests from healthy individuals who came to the hospital for physical examination from January 2014 to January 2019 were retrieved and analyzed. The retrospective clinical evaluation revealed for the first time that circulating TTR was regulated at systems levels and was a systems biomarker for most diseases.

Methods

Study participants

We conducted a retrospective study in the affiliated Hospital of Qingdao University, one of China's largest hospitals. The Ethics Review Committee of Qingdao University Hospital approved this study (QDFYWZLL26222) to retrieve the electronic medical records and laboratory data of serum TTR of both healthy individuals and patients with clinically confirmed diseases in the clinical laboratory of the affiliated Hospital of Qingdao University. All studies were conducted following relevant guidelines /regulations with all participants' informed consent or legal guardians.

Clinical Data Collection

We reviewed more than 48 diseases tested for TTR in the affiliated Hospital of Qingdao University from January 2014 to January 2019. All patients with their primary diagnosis with the specific disease were included in this study. Thus, all diseases included all patients at different stages of disease development with or without medical intervention. To eliminate possible instrumental and human errors, we excluded the top 2.5% and the bottom 2.5% TTR concentration values for each disease. The rest of 95% of data, including 137,305 independent TTR tests in patients suffering 48 types of diseases and 3387 independent TTR tests in healthy individuals, were used for statistical analysis.

Determination Of Serum Ttr Concentrations

Immuno-turbidimetry is a classical method for determining circulating TTR concentrations, a fast, simple, cheap, and accurate assay [48]. Our clinical laboratory uses the " Immuno-turbidimetry" assay routinely for TTR concentration measurement. In this assay, TTR and its corresponding antibodies form an antigen-antibody complex. The measured turbidity of the complex in the reaction mixture is proportional to the circulating TTR concentrations. According to the manufacturer's instructions, TTR concentrations in serum samples were calculated by comparing them with the calibrated TTR concentrations.

Roc Analysis

ROC curves were plotted using SPSS v26 (IBM, Armonk, US). Youden's indices were calculated using ROC curve coordinates 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 median values and mean ± standard deviation (SD) were calculated for each disease and healthy control. A standard t-test was performed to compare the clinical characteristics of subjects in the specific disease and control groups. Median levels of serum TTR concentrations between diseases and healthy controls were compared using 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 statistically significant.

Results

Based on the serum TTR concentration data of the past five years, the number of cases, the mean (standard deviation, SD), median (quartile intervals), and P values (Table 1)(available in Supplement 1)for each of the 48 diseases were compared to healthy controls and summarized in Table 1.

Table 1
Serum TTR concentrations of 48 clinically defined diseases in patients and healthy controls

TTR	# of cases	Mean (SD)	Median (IQR)	*p*-Value
Hepatic Encephalopathy	91	114.3 (89.8)	79.6 (57.7, 128.8)	< 0.001
Cirrhosis	9,298	157.0 (72.2)	151.3 (96.4, 212.4)	< 0.001
Sepsis	114	155.6 (65.0)	154.8 (107.2, 200.6)	< 0.001
Pancreatitis	1,837	178.3 (67.4)	170.6 (126.4, 222.8)	< 0.001
Pancreatic Cancer	1,151	176.5 (72.3)	171.9 (120.9, 229.0)	< 0.001
Liver Cancer	300	184.6 (74.2)	190.4 (120.0, 241.5)	< 0.001
Preeclampsia	993	193.7 (45.4)	196.1 (159.7, 225.0)	< 0.001
Gastric Cancer	13,494	200.5 (63.7)	202.0 (152.0, 247.4)	< 0.001
Chronic Obstructive PD	1,644	207.4 (68.4)	203.1 (156.3, 254.7)	< 0.001
Esophagus Cancer	4,027	209.4 (68.6)	209.7 (156.8, 260.7)	< 0.001
Gastric Ulcer	306	218.1 (70.7)	218.1 (160.8, 270.3)	< 0.001
Acute Myocardial Infarction	2,653	218.4 (64.7)	219.0 (170.6, 266.7)	< 0.001
Brain Trauma	663	226.7 (66.5)	225.4 (176.5, 278.9)	< 0.001
Colon Cancer	6,632	221.9 (74.5)	225.5 (164.1, 278.1)	< 0.001
Myeloproliferative Disorder	1,288	226.0 (91.6)	228.3 (156.5, 296.7)	< 0.001
Bone Cancer	112	231.3 (64.0)	232.7 (184.9, 281.3)	< 0.001
Rectum Cancer	8,508	231.0 (69.8)	233.5 (178.5, 283.2)	< 0.001
Hepatitis	6,728	233.7 (65.0)	236.7 (192.6, 280.0)	< 0.001
Intracranial Hemorrhage	3,857	239.8 (68.0)	240.8 (190.0, 290.5)	< 0.001
Leukemia	5,440	236.7 (80.4)	242.6 (175.1, 298.5)	< 0.001
Encephalitis	560	240.3 (77.7)	243.4 (180.1, 298.0)	< 0.001
Acute Cerebral Infarction	9,658	243.5 (61.4)	244.3 (200.5, 288.2)	< 0.001
Bone Fracture	1,773	247.3 (67.6)	249.0 (199.1, 297.5)	< 0.001
Anemia	2,051	254.1 (89.4)	249.1 (188.6, 321.0)	< 0.001
Lung Fibrosis	338	253.8 (78.5)	250.9 (195.1, 311.7)	< 0.001

Healthy control is bolded to make an easy comparison. SD: standard deviation,

IQR: interquartile range. Chronic Obstructive PD: Chronic Obstructive Pulmonary Disease.

TTR	# of cases	Mean (SD)	Median (IQR)	*p*-Value
Alzheimer's Disease	108	235.5 (74.4)	251.1 (193.3, 275.5)	< 0.001
Breast Lumps	111	261.1 (51.1)	252.2 (227.3, 293.0)	< 0.001
Coronary Heart Disease	22,119	251.2 (64.3)	252.4 (207.7, 297.1)	< 0.001
Rheumatic Arthritis	486	253.2 (62.6)	253.0 (211.3, 295.8)	< 0.001
Asthma	605	253.1 (58.7)	253.0 (211.1, 293.4)	< 0.001
Bladder Cancer	1,071	249.8 (73.2)	253.8 (196.1, 305.3)	< 0.001
Lymphoma	4,555	247.5 (76.9)	255.8 (196.7, 303.9)	< 0.001
Osteoporosis	278	256.8 (56.9)	255.9 (218.1, 296.7)	< 0.001
Lung Cancer	10,932	257.0 (65.2)	259.9 (213.6, 305.4)	< 0.001
Cerebrovascular Disease	4,771	258.9 (59.7)	260.2 (219.9, 300.7)	< 0.001
Cerebral Ischemia	2,279	261.6 (55.0)	260.3 (224.5, 301.0)	< 0.001
Multiple Myeloma	2,329	256.3 (72.0)	260.4 (209.7, 305.6)	< 0.001
Knee-Joint Degenerative Diseases	445	260.5 (50.2)	262.6 (224.4, 297.6)	< 0.001
Bladder Stone	162	262.1 (54.8)	264.7 (225.3, 294.3)	< 0.001
Ovarian Cancer	2,359	261.2 (62.6)	267.0 (224.5, 306.3)	< 0.001
Type 2 Diabetes Mellitus	11,561	266.4 (61.5)	267.8 (225.0, 309.6)	< 0.001
Diabetic Nephropathy	599	268.3 (70.6)	271.0 (219.1, 318.5)	< 0.001
Cerebral Arteriosclerosis	821	269.2 (56.2)	271.4 (232.9, 305.2)	< 0.001
Endometrial Cancer	1,222	272.7 (51.8)	272.4 (238.4, 312.2)	< 0.001
Cervical Cancer	2,274	275.6 (51.8)	276.9 (238.6, 312.7)	< 0.001
Gastritis	3,407	277.1 (58.8)	277.7 (238.1, 318.4)	< 0.001
Psoriasis	145	277.9 (63.5)	277.9 (235.0, 317.9)	< 0.001
Renal Cyst	525	280.5 (52.6)	278.3 (244.5, 317.4)	< 0.001
Necrosis of Femoral Head	173	276.8 (52.4)	278.6 (240.3, 313.5)	< 0.001
Breast Cancer	5,491	281.9 (44.4)	280.0 (250.1, 311.7)	< 0.001
Lupus Erythematosus	1,310	284.6 (78.0)	280.9 (228.0, 334.0)	< 0.001

Healthy control is bolded to make an easy comparison. SD: standard deviation,

IQR: interquartile range. Chronic Obstructive PD: Chronic Obstructive Pulmonary Disease.

TTR	# of cases	Mean (SD)	Median (IQR)	*p*-Value
Ankylosing Spondylitis	102	278.5 (67.5)	285.6 (220.1, 330.7)	< 0.001
Healthy Controls > 65 years old	808	299.5 (50.6)	291.9 (263.1, 331.9)	-
Gout	1,475	296.3 (69.0)	299.4 (248.5, 344.6)	< 0.001
Nephrotic Syndrome	3,838	300.4 (81.6)	299.4 (244.4, 357.8)	< 0.001
Nephritis	2,147	299.9 (69.4)	301.1 (253.5, 348.6)	< 0.001
Kidney Cancer	1,553	309.0 (66.4)	312.6 (269.6, 355.7)	< 0.001
Healthy Controls	**9,473**	**325.1 (55.8)**	**322.3 (283.3, 364.3)**	**-**
Uremia	6,533	337.0 (84.8)	337.2 (277.0, 395.7)	< 0.001
Healthy control is bolded to make an easy comparison. SD: standard deviation,				
IQR: interquartile range. Chronic Obstructive PD: Chronic Obstructive Pulmonary Disease.				

Uremia was the only disease in which the median value of TTR concentrations was higher than that in healthy controls. The median values of TTR concentrations in the rest of 47 different diseases were significantly decreased compared to that in the healthy controls ($p < 0.001$, Table 1). Among the 47 diseases, patients diagnosed with hepatic encephalopathy had the lowest median value of TTR concentrations, followed by patients suffering cirrhosis, sepsis, pancreatitis, pancreatic cancer, liver cancer, preeclampsia, and gastric cancer, respectively. (Table 1).

Receiver operating characteristic curve (ROC) analysis was then performed for all 48 different types of diseases. Based on the ROC analysis (Fig. 1), the area under the curve (AUC), accuracy, sensitivity, and specificity for all diseases were summarized in Fig. 2. Among the 48 diseases, 40 of them had AUCs above 0.60, ranging from 0.60 to 0.97. TTR served as the best biomarker for sepsis with the AUC, sensitivity, specificity of 0.97,0.93, and 0.89, respectively, followed by cirrhosis, pancreatitis, live cancer hepatic encephalopathy based on AUC values (Fig. 2). Interestingly, TTR had the lowest AUCs for all female-related cancers, which included ovarian cancer (0.66), endometrial cancer (0.63), cervical cancer (0.60), and breast cancer (0.59), compared to that in other types of cancers except for kidney cancer. The AUC values were below 0.60 for the following diseases, i.e., uremia, nephrotic syndrome, lupus erythematosus, nephritis, kidney cancer, azotemia, and aplastic anemia. However, all the diseases with lower AUC values were associated with the abnormal shape of the ROC curves, as shown in Fig. 1B and 1C.

We noticed that certain diseases had high specificity (0.90-1.00) but low sensitivity (0.08–0.55) when TTR was served as a biomarker (Fig. 2). Such diseases were kidney-related diseases including diabetic nephropathy, kidney cancer, nephritis, nephrotic syndrome, and azotemia; female-associated cancers including cervical cancer and ovarian cancer; and blood-related cancer and diseases including leukemia, myeloproliferative disorder, and anemia; and autoimmune diseases including rheumatoid arthritis,

systemic lupus erythematosus. Most of these diseases had either S- or reversed S-shaped ROC curves (Fig. 1B and 1C).

The serum TTR concentration distributions of 48 diseases were plotted to show the relationships between different ROC curves and the AUC values among different diseases (Fig. 3). Our data analysis contained 95% of the data collected during the past five years by excluding 2.5% of the patients with the highest and lowest serum TTR values, respectively, to eliminate potential human and instrumental errors. The lowest value (2.5 percentile), the first quartile (25 percentile), the median quartile (50 percentile), the third quartile (75 percentile), and the maximum value (97.5 percentile) were shown for each of the 48 diseases.

Among all diseases, the serum TTR concentrations in patients suffering hepatic encephalopathy had the lowest median level (50 percentile) and the lowest serum TTR 2.5%, 25%, 75%, and 97.5% values (Fig. 3). The median value of TTR concentrations in patients suffering hepatic encephalopathy was about four times lower than that in the healthy controls (322.3 mg/L vs. 79.6 mg/L), which was followed by a 2-fold decrease in liver cirrhosis (322.3 mg/L vs. 151.3mg/L) and a 2-fold decrease in sepsis (322.3 mg/L vs. 154.8 mg/L), a 1.9-fold decrease in pancreatitis (322.3mg/L vs. 170.6 mg/L) and pancreatic cancer, and 1.7-fold decrease (322.3 mg/L vs. 190.6 mg/L) in liver cancer compared to that in healthy controls (322.3 mg/L). In contrast, all kidney-related diseases had relatively higher median values of TTR concentrations compared to other diseases. Moreover, kidney- and blood-related diseases had the broadest TTR concentration ranges. The above data suggested that the liver, pancreas, kidney, and blood were the major sites that caused up- and down-regulations of circulating TTR concentrations.

TTR concentration distributions in 6 categories of diseases and healthy controls were plotted in Fig. 4. Among the six categories of diseases, liver-related diseases had the lowest, whereas kidney-related diseases had the highest median TTR values. Notably, the two aging-related fetal diseases, cancers and vascular diseases, had lower TTR median values than those in autoimmune and kidney-related diseases. Moreover, blood-, autoimmune-, and kidney-related diseases had one common characteristic: almost doubled lowest to highest TTR concentration ranges compared to that in the healthy controls. Furthermore, the 97.5 percentile TTR values for blood-, autoimmune-, and kidney-related diseases were exceeded that in the healthy controls, which indicated that TTR concentrations were over up-regulated in blood-, autoimmune-, and kidney-related diseases.

We further analyzed six statistical characteristics of serum TTR concentrations, including mean, standard deviation, 2.5, 25, 50, 75, 97.5 percentiles. The 48 diseases were divided into six main categories: cancers, autoimmune diseases, vascular diseases, blood-related diseases, and kidney diseases. The color-coded results of the major component analysis for 48 diseases were shown in Fig. 5.

Indeed, clustering of the same category of diseases was observed. Kidney diseases, i.e., nephritis, diabetic nephropathy, azotemia, nephrotic syndrome, and uremia, were located at the lower-left part of Fig. 5. Cancers and vascular diseases were around the center of Fig. 5. The autoimmune diseases were at the

downside of Fig. 5. Interestingly, liver cancer, pancreas cancer, liver- and pancreas-related diseases, and other critical illnesses with the lowest TTR median values were scattered at the far-right side of Fig. 5.

Discussion

Changes in circulating TTR concentrations have been reported during the past [12, 13, 16, 18, 27, 28, 31, 32, 39, 43, 49–54] one disease at a time. We concluded that TTR had the typical characteristics of a systems biomarker and was regulated at the systems level by comparing the serum TTR concentrations in 48 different diseases for the first time. The evidence we presented in the current study including 1. Down-regulated TTR concentrations were typical in all human diseases even though the liver was the primary TTR production site (Table 1); 2. ROC analyses indicated that circulating TTR served as decent biomarkers for 40 diseases (AUCs > 0.60); 3. Over up- and down-regulations of TTR concentrations explained the abnormal ROC curves and the low AUCs observed in the kidney- and blood-related diseases (Figs. 1, 2, 3&4); 4. Critical illnesses, such as hepatic encephalopathy, sepsis, and preeclampsia, had the lowest TTR concentrations, and TTR served as excellent systems biomarkers for these diseases based on their AUC values; 5. Significantly decreased TTR concentrations in liver-related diseases further confirmed that the liver was the site of TTR synthesis; 6. Chronic fatal diseases, such as cancers and vascular diseases, had the narrowest TTR concentration ranges, whereas kidney-, autoimmune- and blood-related diseases had the broadest TTR concentration ranges (Figs. 3, 4 &5).

Circulating TTR has been the most used circulating biomarker to detect patients' nutritional status and monitor patients' response to nutritional therapy[45]. However, our data and other published results indicated that circulating TTR might reflect the types and severities of illnesses more than nutritional status[45]. In addition, there is no evidence that raising circulating TTR concentrations through aggressive nutritional support improves outcomes[9]. Thus, defining TTR as a systems biomarker instead of a nutritional biomarker might be better fitted for systems care of patients in the future.

Circulating TTR has an average half-life of 2.5 days[19], and its concentrations are affected by age, sex, diet, lean body mass, and disease state are [55]. The overall speed of TTR biosynthesis and turnover/degradation determines its circulating concentrations. Published data showed that TTR biosynthesis decreases in liver diseases, and TTR concentrations are reduced during kidney dialysis, acute blood loss, hunger, hyperthyroidism, nephrotic syndrome, hyperglycemia, and protein loss bowel disease [45, 56]. TTR biosynthesis is up-regulated by corticosteroids, non-steroidal anti-inflammatory drugs, chronic renal failure, and tubular injury. Cytokines, such as interleukin-6, decrease the biosynthesis of the negative acute-phase proteins, including TTR but increase the biosynthesis of positive acute phase reactions C-reactive protein, serum amyloid-A and α1-antitrypsin[21, 45, 57, 58]. The multi-level regulations of TTR biosynthesis and turnover/degradation further supported our notion that TTR was a systems biomarker.

Our results indicated that the liver was the primary site for TTR biosynthesis. In contrast, the kidney might be the major site for TTR turnover/degradation based on the data shown in Figs. 2&3, consistent with the

published results in a rat model[59] and patients suffering kidney diseases[60]. We also noticed that blood-related diseases had the broadest TTR concentration ranges, which suggested that acute inflammation, infection, malignancy, or medical intervention in blood-related diseases could cause over- and down-regulations of TTR concentrations. Systems swings between catabolic and anabolic phases might underlie such observations.

Conclusions

By systematically comparing TTR concentrations in 48 diseases and healthy controls, the overall results indicated that TTR was a valid candidate of systems biomarkers. The liver, kidney, blood, and pancreas were the major organs involved in regulating circulating TTR concentrations.

Declarations

Ethics approval and consent to participate

The Hospital Ethics Review Board of Qingdao University approved the current study. All research was performed following relevant guidelines/regulations, and

informed consent was obtained from all participants or their legal guardians.

Consent for publication

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

Availability of data. All data files are available upon request. Correspondence and

requests for the data files should be addressed to H.C and L.Z.

Data analysis. Correspondence and requests for detailed data analysis should be

addressed to H.C., 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), the Natural Science Foundation of Shandong province (ZR2020QH082), Youth fund of Affiliated Hospital of Qingdao University (Grant No. 3105), and “Clinic+X” project of Affiliated Hospital of Qingdao University (Grant No. 3442).

Author contributions All authors had full access to all data in the study and takes responsibility for the integrity of the data and accuracy of the data analysis. Concept and design: Z.W., M.Z., L.Z., Y.G., and H.C;

acquisition, analysis or interpretation of data: Z.W., M.Z., Y.Z., Y.H., F.Q., C.B., L.Z., Y.G., and H.C; statistical analysis: Z.W., M.Z., Y.Z., Y.H., Y.G., L.Z., and H.C; obtaining funding: L.Z. and Y.G.

Acknowledgments We thank all the staff and patients in our hospital who made

the current study possible. We are also grateful to all at Systems Biology & Medicine Center for Complex Diseases who have helped the project in various ways.

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Figures

Figure 1

Circulating TTR as a biomarker for 48 types of human diseases assessed by receiver operating characteristic (ROC) curve analysis. A. The diseases with a C-shaped ROC curve with no data points crossing the dashed diagonal line; B. Five diseases with an S-shaped ROC curve crossing the dashed diagonal line. C. Two diseases with reversed S-shaped ROC curve crossing the dashed diagonal line. AUC: area under the curve.

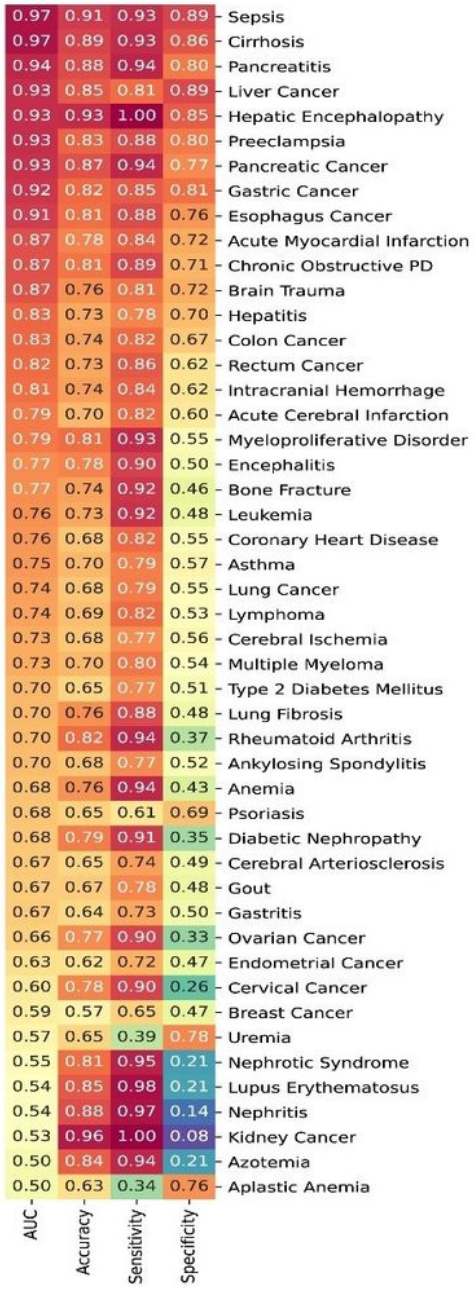

Figure 2

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

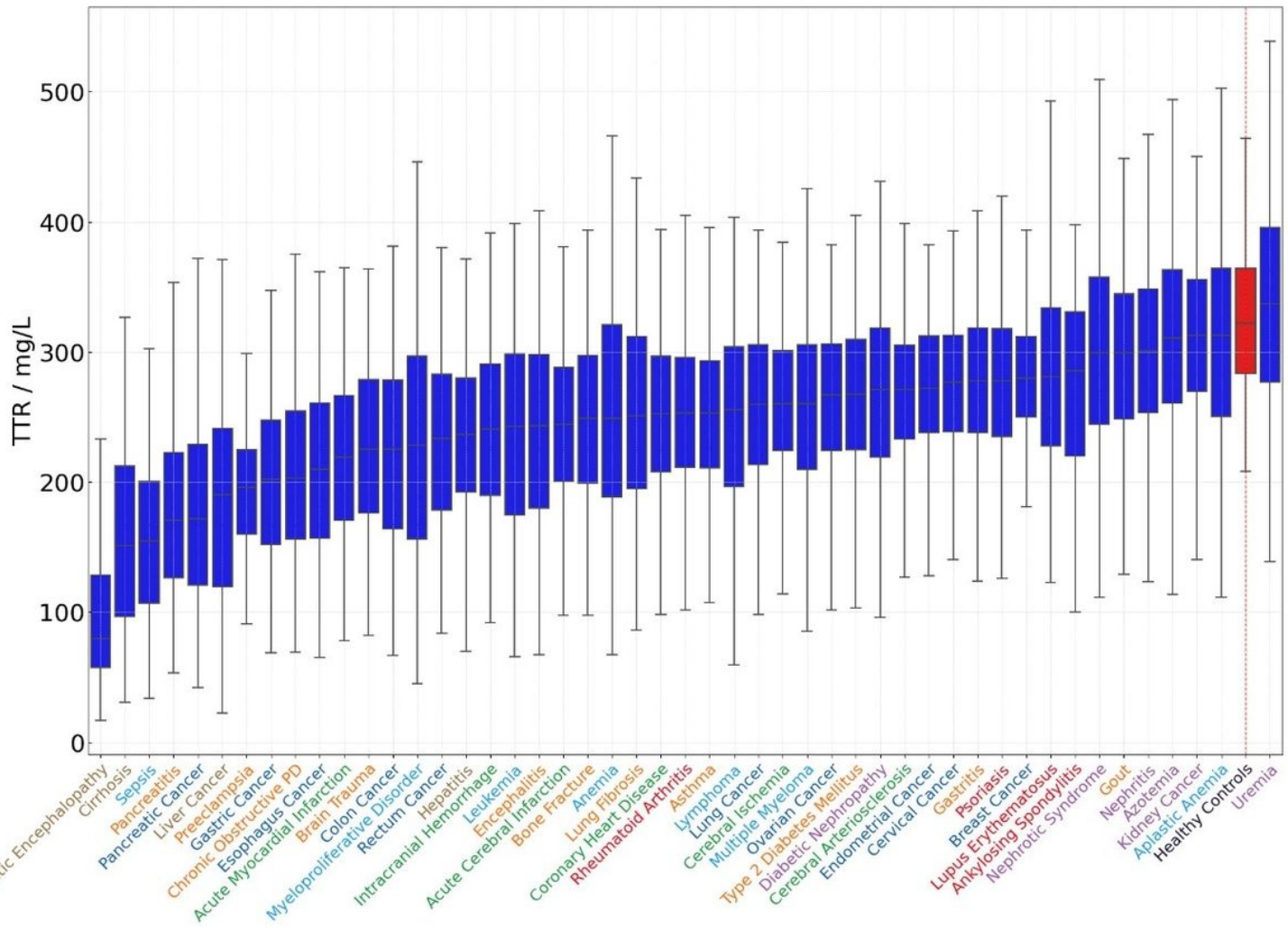

Figure 3

serum TTR concentrations in 48 different diseases and healthy controls. The data of serum TTR are arranged in an ascending order according to the median values. Chronic Obstructive PD: Chronic Obstructive Pulmonary Disease.

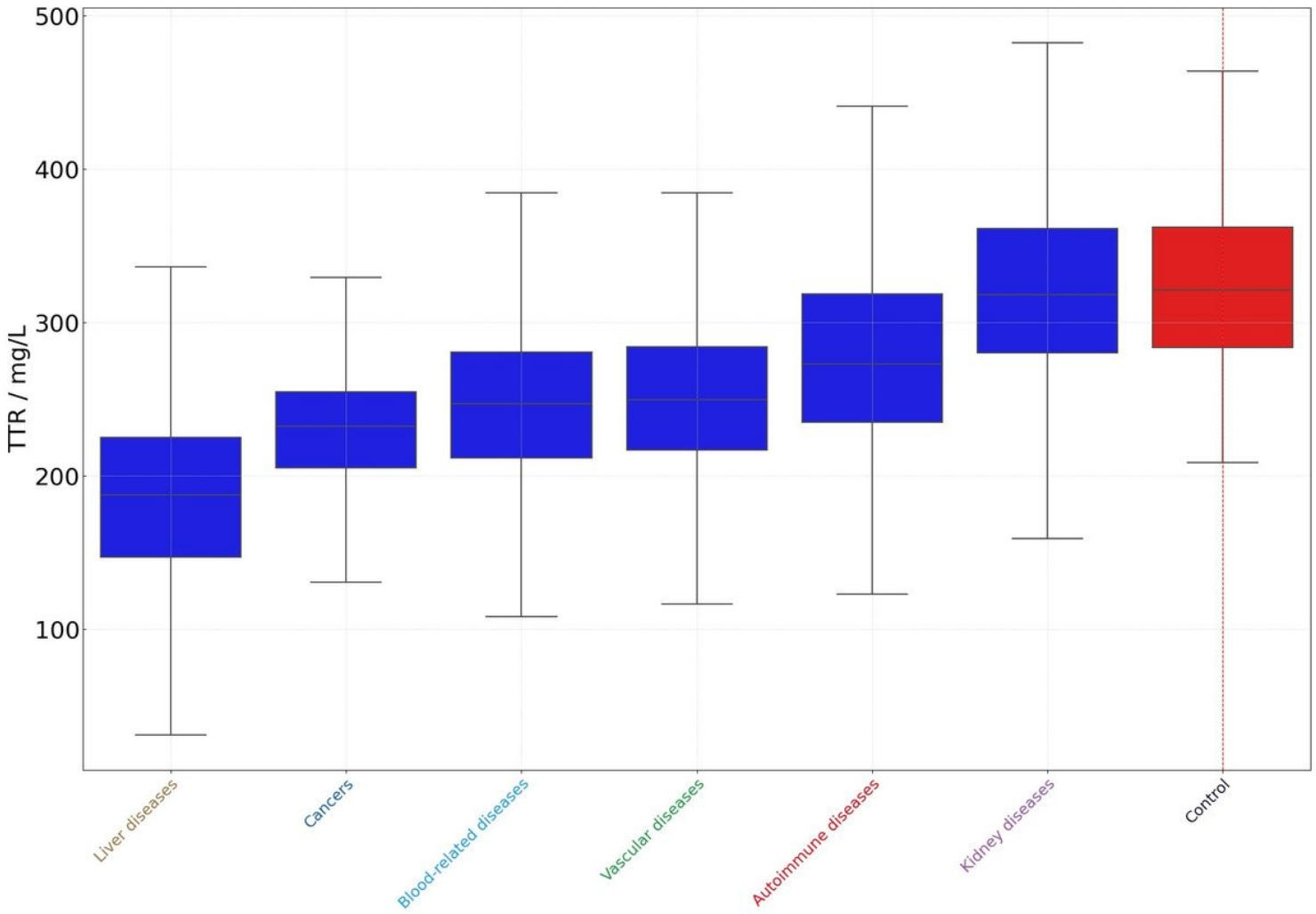

Figure 4

TTR concentration distributions in 6 categories of diseases and healthy controls. Liver diseases included hepatic encephalopathy, cirrhosis, and hepatitis.

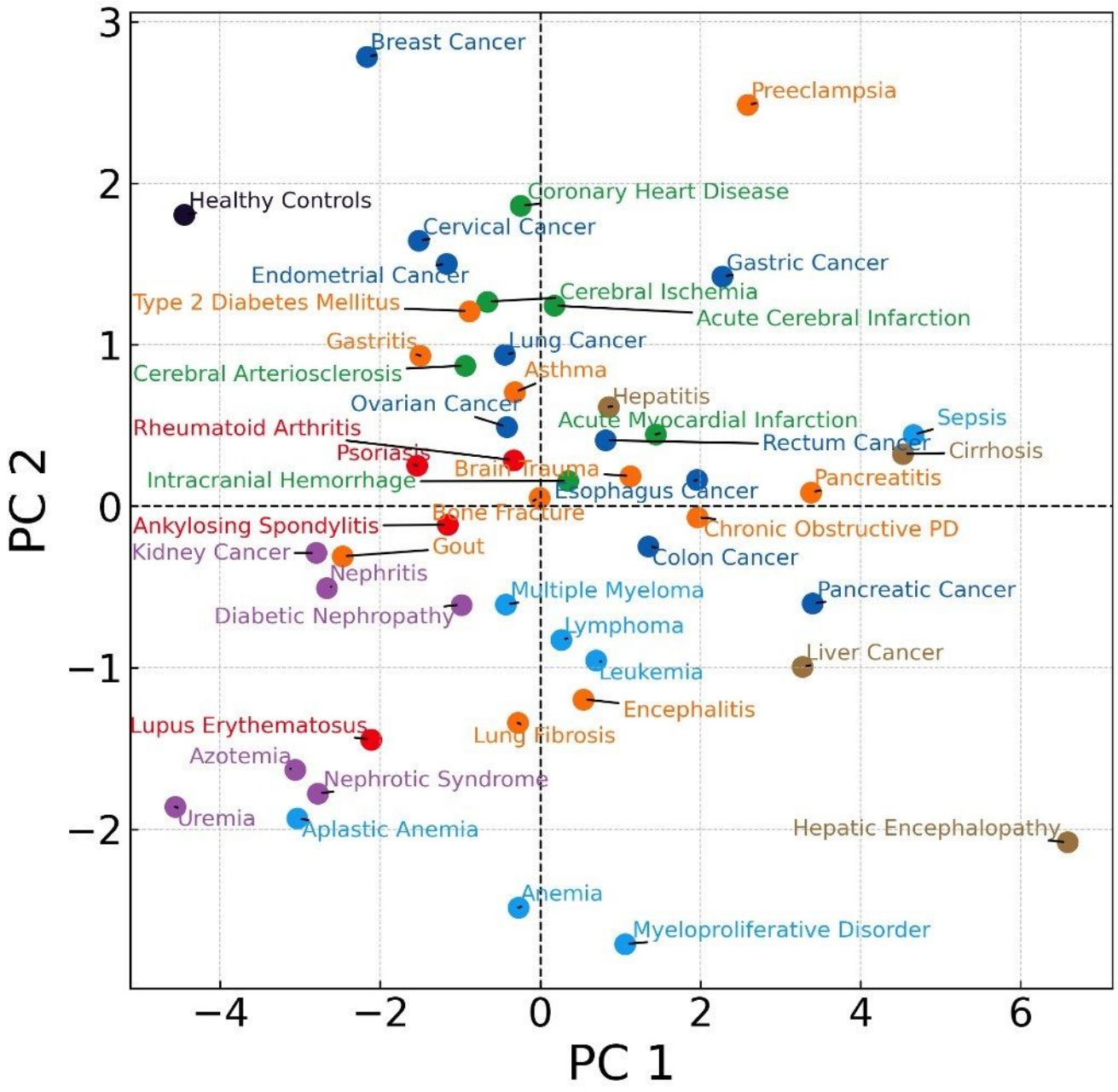

Figure 5

TTR in the same category of diseases was clustered together based on principal component analysis. Forty-eight diseases were divided into six main categories: liver diseases, cancers, autoimmune diseases, cardiovascular and cerebrovascular diseases, blood-related diseases, kidney diseases, marked with brown, dark blue, red, green, light blue, and purple, respectively. The statistical characteristics of circulating TTRs in 48 diseases were quantitatively analyzed, including median, mean, standard

deviation, and the values of minimum, maximum, 25, 50, and 75 percentiles. The obtained statistical features of all 48 diseases were further decomposed into two principal components and presented.