## Original Research Article

# Association between cystatin C and the interaction of pulmonary tuberculosis with chronic diseases

**Huan Wu[1], Jialing Xu[1], Yufeng Wen[1]\*, Xia Dan[1], Mengxue Liu[1], Jun Ma[1], Zewei Wu[1] and Hua Dong[2]**

[1]School of Public Health, [2]School of Clinical Medicine, Wannan Medical College, Wuhu, Anhui, PR China

*For correspondence: **Email:** wyf2015w@sina.com; **Tel:** +86 0553 3932059

## Abstract

**Purpose:** To determine the association between Cystatin C (Cys C) levels and the interaction of pulmonary tuberculosis (PTB) with chronic diseases (CD).
**Methods:** Participants (n = 356) were selected randomly from The First Affiliated Hospital of Wannan Medical College, China, and divided into 4 groups: normal control group (n = 80), PTB group (n = 98), chronic disease group (n = 146), and PTB combined with chronic disease group (PTB+CD, n = 31). The investigation included information on demographics and analysis of blood samples for Cys C, liver function, renal function, blood glucose and other biochemical indices.
**Results:** The highest level of Cys C was obtained in PTB + CD group. Before and after adjusting eGFR, there was no association between Cys C and PTB or/and chronic disease. However abnormal levels of Cys C were significantly higher in PTB+CD group after adjusting eGFR (OR = 4.014, p = 0.0125).
**Conclusion:** Higher levels of Cys C may be associated with chronic diseases co-existing with PTB.

**Keywords:** Cystatin C, Pulmonary tuberculosis, Chronic diseases, Inflammation

## INTRODUCTION

Pulmonary tuberculosis (PTB) is a serious threat to human health and there are approximately one third of the world's population are latently infected *M. tuberculosis* [1]. Despite expansion of directly observed treatment short course strategy and substantial investments aimed at improvements in treatment completion rates, inaccurate diagnosis remains one of the biggest obstacles to global tuberculosis control. Due to the high prevalence of cardiovascular diseases (CVD), public health programs are confronted with the challenge of epidemic of CVD and overlapping tuberculosis [2].

Cys C is produced by all nucleated cells and removed from the bloodstream by glomerular filtration [3]. It is recognized as a marker for detecting glomerular function chronic kidney disease (CKD), acute kidney injury and related sequelae [4-6]. Studies also show that Cys C is related to cardiovascular diseases, such as cardiac accident and coronary artery disease [7]. Cys C is implicated in chronic low-grade inflammation in the pathogenesis of atherosclerosis. A causal link between increased Cys C concentrations and impaired cardiovascular outcome has been proposed [5,6,8].

Recent studies found that serum Cys C may affect the stability of plaques among patients with normal kidney function, and may be associated with increased carotid plaque burden [9]. It has also been reported that Cys C levels in diabetic

patients are higher than the levels in healthy non-diabetics [10]. The pathogenic role of *Mycobacterium tuberculosis* is also related to inflammation caused by proliferation of bacteria, as well as body impairment produced due to delayed type hypersensitivity reaction [11]. An important survival mechanisms of *Mycobacterium tuberculosis* is preventing contact with active cathepsins by inhibiting phagosome maturation [12]. It is known that some cathepsins (cathepsin B, D and S) interact and contribute to the destruction of invading microorganisms [13]. Consistent with the role of Cys C as a natural inhibitor of cathepsins, cystatin is down-regulated in macrophages infected with *Mycobacterium tuberculosis*. Indeed, treatment with cystatin led to a significant 5-fold increase in *Mycobacterium tuberculosis* survival rate after 24h of infection in resting macrophages [14]. Down - regulation of cathepsin in macrophages induced by Mycobacterium tuberculosis. The increase in gene expression most of the cystatins was observed, although there is an increased cathepsin gene expression, a concomitant increment of Cys C should impair their activities in IFNγ- activated macrophages [14].

However, there are limited studies on the levels of Cys C in patients with PTB combined with chronic diseases (hypertension, diabetes and cardiovascular disease). In this context, we propose a hypothesis that *Mycobacterium tuberculosis* patients with cardiovascular diseases may have higher level of Cys C. Therefore in this study, the Cys C levels of patients with PTB, chronic diseases, and PTB combined with chronic diseases were investigated.

## METHODS

### Study subjects

All participants in the control and case groups were randomly selected from the First Affiliated Hospital of Wannan Medical College. Healthy individuals without PTB and chronic diseases (hypertension, diabetes and cardiovascular disease) were in the control group (NC, $n = 80$). The case groups included PTB ($n = 98$), PTB with chronic diseases group (PTB + CD, $n = 31$) and chronic diseases group (CD, $n = 146$). All participants satisfied these conditions: (1) no history of liver dysfunction, and (2) no tumor history. The study was conducted in compliance with Helsinki guidelines of the Helsinki Declaration of World Medical Association [15], and according to the protocol approved by Medical Ethics Committee of Wannan Medical College (approval no. 2014005). All participants

were told the study objective and they gave verbal informed consent prior to commencement of the investigation.

### Assay of Cys C and other biochemical indices

Venous blood samples were taken from the participants after overnight fast, and sera were separated after centrifugation at room temperature. The serum samples obtained were assayed for Cys C, liver function parameters, renal function parameters, blood glucose and other biochemical indices using latex enhanced transmission immunoassay.

Estimated glomerular filtration rate (eGFR: $ml/min/1.73\ m^2$) was calculated by Modification of Diet in Renal Disease (MDRD) formula (Eq 1) [16].

$$eGFR = 175\times (Cr/88.41)^{-1.154} \times Age^{-0.203}\ (\times 0.742\ \text{if female}) \cdots\cdots (1)$$

Serum Cys C was determined by immunoturbidimetric assay. Normal reference rangs of Cy C are 0.54 - 1.14 and 0.63 - 1.25 (mg/L) in females and males, respectively.

Serum creatinine (Cr) was determined by isotope dilution mass spectrometry (IDMS) method. The normal reference ranges of creatinine are 44.00 - 80.00 and 62.00 - 106.00 (μmol/L) in females and males, respectively.

Blood urea nitrogen (BUN) was determined by urease method, and its normal reference value is 2.14 - 8.21 mmol/L.

### Diagnostic criteria for PTB and other diseases

#### *PTB*

These included confirmed cases such as sputum smear positive PTB cases, only-sputum culture-positive PTB cases and cases with pulmonary lesions of TB that have been confirmed by pathological examination [17]. Smear positive PTB met one of the following three criteria: Two sputum specimens directly smear positive for anti-acid bacteria microscopic examination; a sputum smear positive direct microscopic examination, and lung imaging consistent with active PTB imaging manifestation and one positive direct smear microscopy result plus one positive sputum culture for *M. tuberculosis;* Only-culture positive PTB met two criteria namely: negative sputum smears and lung imaging consistent with active PTB imaging

manifestations plus one positive sputum culture for *M. tuberculosis*.

### Hypertension

Systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg, or if participant was at that time on medication to control blood pressure [18].

### Diabetes

Fasting blood glucose ≥ 7.1 mmol/L or if patient was at that time on drug to control blood glucose [19].

### Carotid artery disease

Near and far walls of the common carotid, and internal carotid artery on both sides were scanned for hemodynamics with 5 -13 MHz linear-array probes (Aloka - a7 and Aloka - a10, Japan; Philips - IU22, America) [20].

### Coronary heart disease

Coronary angiography was based on standard Judkins technique [21] by cardiologists who have no clue the ultrasonography results.

### Statistical analysis

Data were analyzed statistically using SPSS 18 software. Differences in demographic characteristics were analyzed by chi-square test, while ANOVA was used to analyze differences in age, Cys C content, BUN and eGFR among the four groups. For unadjusted and adjusted eGFR, non-conditional logistic regression analysis was used to analyze the relationship between chronic disease, PTB and Cys C. The relationship between Cys C and eGFR was analyzed with linear regression analysis. A two-tailed *p* value <0.05 was considered statistically significant.

## RESULTS

### Characteristics of subjects

Table 1 shows that Cys C and eGFR were significantly different among control group, PTB group, chronic diseases group and PTB + CD group. The levels of Cys C were highest in the PTB + CD group, while levels of eGFR were lowest in this group (PTB + CD group). There were no significant differences in sex, age and BUN among the four groups.

### Relationship between chronic disease and pulmonary tuberculosis, and their interaction with Cys C

Table 2 shows that Cys C levels were not significantly related to chronic disease or PTB regardless of whether eGFR was adjusted or not, but Cys C level in the PTB +CD group was 4.014 times ($x^2$ = 6.2401, *p* = 0.0125) higher than that of the control group after adjusting eGFR.

### Relationship between Cys C and eGFR

The relationship between Cys C and eGFR is depicted in Figure 1. Cys C and eGFR were negatively correlated in the four groups: Cys C increased with decrease in eGFR.

**Table 1:** Subject characteristics of the four groups

| Variable | | PTB (n=98) | CD (n=146) | PTB+CD (n=31) | NC (n=80) | $X^2$/F | P |
|---|---|---|---|---|---|---|---|
| Sex | Female | 27(27.55%) | 40(27.40%) | 9(29.03%) | 22(27.50%) | 0.0353 | 0.9983 |
| | Male | 71(72.45%) | 106(72.60%) | 22(70.97%) | 58(72.50%) | | |
| Age | | 53.70±18.85 | 53.54±15.90 | 53.52±17.84 | 59.00±11.60 | 2.3200 | 0.0750 |
| Cys C | | 1.06±0.29 | 1.01±0.26 | 1.35±0.53 | 1.13±0.33 | 9.7100 | <0.0001 |
| BUN | | 5.63±2.51 | 5.56±1.41 | 6.97±7.62 | 5.89±1.91 | 2.0200 | 0.1110 |
| eGFR | | 107.85±31.47 | 101.52±24.51 | 90.09±25.81 | 95.52±21.41 | 3.9600 | 0.0086 |

*Cys C: cystatin C; BUN: blood urea nitrogen; CRP: C-reactive protein; eGFR: glomerular filtration rate*

**Table 2:** Logistic analysis of chronic disease, tuberculosis and their interaction with the logistic analysis of cystatin C

| Variable | Unadjusted | | | Adjusted | | |
|---|---|---|---|---|---|---|
| | $x^2$ | P | OR(95%CI) | $x^2$ | P | OR(95%CI) |
| CD | 1.0655 | 0.3020 | 0.684(0.333-1.406) | 0.3022 | 0.5825 | 1.809(0.380-1.723) |
| PTB | 1.3941 | 0.2377 | 0.623(0.284-1.366) | 0.0229 | 0.8798 | 0.938(0.407-2.161) |
| PTB+CD | 3.6187 | 0.0571 | 2.489(0.973-6.368) | 6.2401 | 0.0125 | 4.014(1.349-11.942) |
| Intercept | 10.4948 | 0.0012 | | 9.2737 | 0.0023 | |

*Adjusted factor: eGFR*
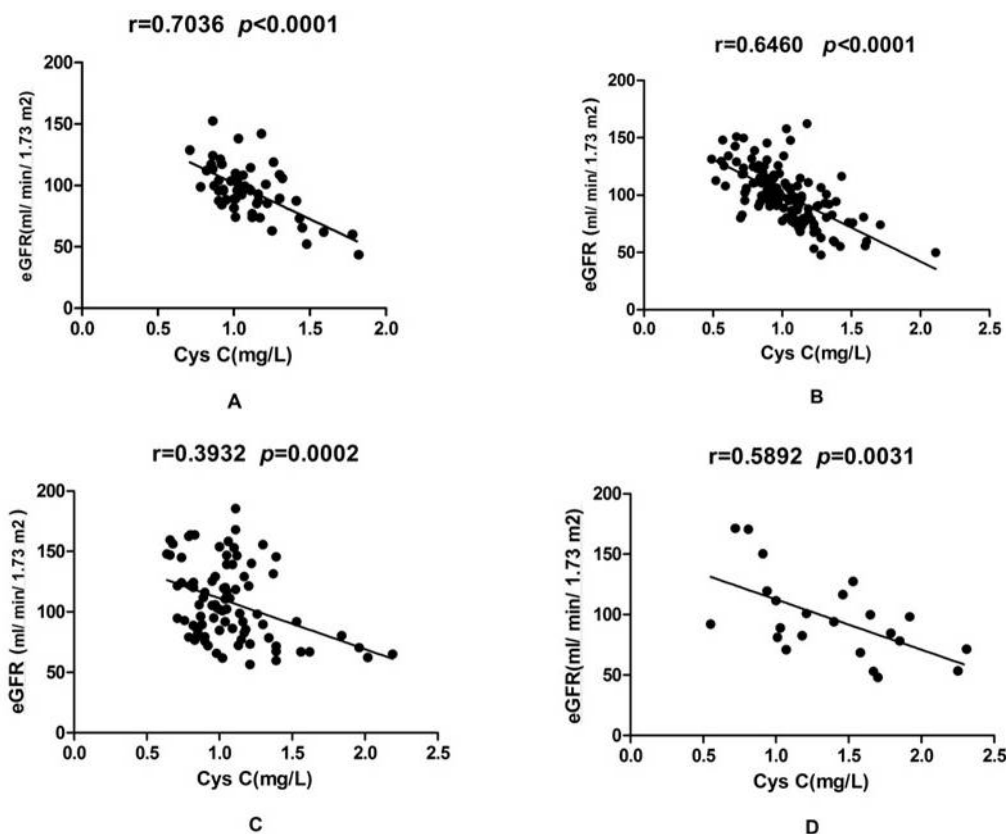
**Figure 1:** Relationship between Cys C (mg/L) and eGFR (ml/min/1.73 m$^2$). Cys C was negatively correlated with eGFR; A: control group (NC); B: chronic diseases group (CD); C: PTB group; D: PTB+CD group.

## DISCUSSION

The prevention and treatment of tuberculosis have become more difficult with global increase in drug-resistant tuberculosis. With lifestyle transformations and rapid development of the global economy, the incidence of chronic diseases has continued to grow rapidly, with more tuberculosis patients having chronic diseases. Thus it is necessary to improve prevention and treatment strategies for tuberculosis patients with chronic diseases.

This study shows the association between Cys C and interaction of tuberculosis with chronic diseases. The levels of Cys C were highest in the PTB + CD group. Cys C levels were not significantly related with chronic disease or PTB regardless of whether eGFR was adjusted or not. Cys C level in the PTB +CD group was 4.014 times higher than that of the control group after adjusting eGFR Cys C increased with decrease in eGFR in the four groups.

Although atherosclerosis is a complex process, Genome-Wide Association Study (GWAS) research and candidate gene studies support the involvement of inflammatory mechanisms in the pathogenesis of atherosclerosis. Recently, GWAS identified a number of coronary heart diseases and inflammation-associated genes. Recent studies also showed that immune and inflammatory responses and endothelial dysfunction are key links in the pathogenesis of hypertension [22]. In addition, the inflammation hypothesis of diabetes suggests that a low degree of natural immunity as well as inflammatory factors are associated with diabetes [23]. There is a competition between pro- and anti-inflammatory signals in PTB. Ingestion of intracellular bacteria by macrophages and dendritic cells initiates the first important immune responses. Infected dendritic cells can express IL-12, TNF-α and IL-10 cytokines [24], while macrophages produce mainly TNF-α, IL-10 and a small amount of IL-12 [25]. These provoke a series of inflammatory reactions.

From the analysis of results obtained in this study, chronic diseases combined with PTB might result in higher levels of Cys C, probably due to the fact that inflammation is involved in chronic diseases and TB. Cys C and eGFR were negatively correlated. Since Cys C is a cysteine protease inhibitor, it is more reliable than

creatinine and BUN in reflecting endogenous glomerular filtration rate. Renal impairment may lead to elevated Cys C, and since it involves inflammation, it may have a compounding effect.

## CONCLUSION

The results obtained in this study suggest that Cys C is not only a sensitive indicator of renal function but also an independent factor for predicting the risk of pulmonary tuberculosis associated with some chronic diseases (hypertension, diabetes and cardiovascular diseases). Therefore, Cys C is likely to be an indicator of inflammation.

## DECLARATIONS

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### *Conflict of Interest*

No conflict of interest associated with this work.

### *Contribution of Authors*

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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