

RESEARCH

Open Access



# Cystatin C as a predictor for carotid artery steno-occlusive disease in non-cardioembolic ischemic stroke

Maha Atef Zaki<sup>1</sup>, Sahar Abd Atty<sup>2</sup>, Alshaimaa M. Aboul fotouh<sup>1\*</sup> and Nancy Samir Wahdan<sup>1</sup>

## Abstract

**Background:** Stroke is the third leading cause of death in developed nations. Ultrasonography (US) of the carotid arteries is the modality of choice for diagnosis and monitoring of cases of atheromatous disease. Cystatin C (CysC) is a risk factor for cerebrovascular and cardiovascular disease.

**Objectives:** To detect the association of serum CysC levels with acute ischemic stroke, the correlation of its levels with stroke severity, and the association between CysC levels with extra- and intracranial stenosis.

**Methods:** Case-control study was conducted on 66 subjects, 33 patients within the first week of stroke onset with age range from 40 to 75 years, and 33 control healthy subjects with matched age and sex. All subjects underwent complete neurological examination, Extra- and transcranial duplex, CT brain, routine laboratory work-up, and serum CysC.

**Results:** Serum CysC levels were significantly raised in patients with acute ischemic stroke compared to controls. CysC levels were higher in patients with extracranial stenosis than in those with intracranial stenosis. Moreover, extracranial stenosis > 50% has higher levels of CysC. Serum CysC level showed a significant correlation with National Institutes of Health Stroke Scale (NIHSS) and intima-media thickness (IMT).

**Conclusion:** Serum CysC can be used as a marker for acute atherosclerotic ischemic stroke and stroke severity. High-grade extracranial stenosis has the highest level of CysC.

**Keywords:** Acute ischemic stroke, Cystatin C, Extra- and transcranial duplex ultrasonography

## Introduction

About 25–30% of all cerebral ischemic events are caused by large vessel atherosclerosis. CysC may contribute to the process of carotid atherosclerosis via chronic inflammation [1]. Ultrasonography (US) of the carotid arteries is the modality of choice for diagnosis and monitoring of cases of atheromatous disease [2].

The aims of our work are to evaluate the association of serum CysC level within the first week of the stroke and to correlate its level with the severity of the stroke according to NIHSS and to determine whether CysC levels were associated with extra and transcranial large artery stenosis (LAS).

## Patients and methods

In this case-control study, we chose 66 subjects who were divided into group A (study group) and group B (control group). Group A includes 33 patients within 1 week from the onset of ischemic stroke, 11 (33.3%) patients have extracranial stenosis, 11 patients with intracranial stenosis, and 11 patients with mixed intra- and extracranial stenosis, and they were recruited from the neurology in-patient stroke unit of Kasr El-Aini Teaching Hospital. Group B includes 33 healthy subjects with matched age and sex. The study was performed between November 2017 and May 2018. Inclusion criteria were as follows: age range 40–75 years old, both sex, intracranial and or extracranial stenosis by the duplex US, and we excluded patients < 40 years or > 75 years, patients with intracranial hemorrhage, potential cardiac embolic sources, malignancy, creatinine > 1.5 mg/dl, hypothyroidism, and chronic inflammatory diseases. All

\* Correspondence: [moyamoya15@yahoo.com](mailto:moyamoya15@yahoo.com)

<sup>1</sup>Department of Neurology, Faculty of Medicine, Cairo University, Al Orman Giza Governorate 12613, Giza, Egypt

Full list of author information is available at the end of the article

patients were subjected to full history, general, and neurological examination including NIHSS, extra-, and transcranial neurosonological assessments using Philips IU22 duplex machine software, version 2.0.13, USA 2012, and routine laboratory investigations and serum level of CysC using Siemens N Latex Cystatin C kit on BN ProSpec Nephelometer used in BN ProSpec Company from Siemens Healthcare Diagnostics, product GmbH 35041, Marburg/Germany. Brain computed tomography (CT) with GE multislice 64, 32 detector\*2, USA, 2015, and echocardiography with Samsung Medison HS60, Korea.

### Statistical analysis

Data were coded and entered using the statistical package SPSS, version 25; SPSS Inc., Chicago, IL, USA. Data was summarized using mean and standard deviation for quantitative variables and frequencies (number of cases) and relative frequencies (percentages) for categorical variables. Comparisons between groups were done using analysis of variance (ANOVA) with multiple comparisons post hoc test or unpaired test in normally distributed quantitative variables while non-parametric Kruskal-Wallis test and Mann-Whitney test were used for non-normally distributed quantitative variables [3]. For comparing categorical data, chi-square ( $\chi^2$ ) test was performed. Exact test was used instead when the expected frequency is less than 5 [4]. Correlations between quantitative variables were done using Spearman correlation coefficient [5]. A multi-variate linear regression model was conducted. *P* values less than 0.05 were considered as statistically significant.

## Results

### Demographic and clinical data

Mean age of group A was ( $56.76 \pm 11.12$ ), and out of 33 patients, 20 were males (60.6%) and 13 were females (39.4%). The mean age of group B was ( $47.33 \pm 5.19$ ) with 25 males (75.8%) and 8 were females (24.2%).

Within group A, 19 patients were diabetic (57.6%), 22 patients were hypertensive (66.7%), and 14 patients were smokers (42.4%) compared to 3 smokers (9.1%) only in group B.

Patients NIHSS range from 4 to 20 with a mean of  $10.52 \pm 4.29$ .

Twenty-one out of 33 patients had branch MCA infarction (63.6%) in CT brain, 8 have a total MCA infarction (24.2%), 3 have lacunar infarction (9.1%), and only 1 patient was had MCA and PCA infarction (3.0%). Among 33 patients in group A, 24 (72.7%) had increased IMT.

In the current study, 9 out of 11 patients with extracranial stenosis had stenosis > 50% while 6 out of 11 patients with intracranial stenosis had stenosis > 50%, and only 4 patients with mixed intra- and extracranial stenosis had stenosis > 50%.

In group A, 22 patients had high CysC level (66.7%), but in group B, all had normal CysC level sensitivity 66% and specificity 100%.

TLC was high in 2 patients in group A (6.1%), lipid profile was high in 12 (36.4%), and coagulation profile, renal, hepatic function, and electrolyte level were normal in both groups.

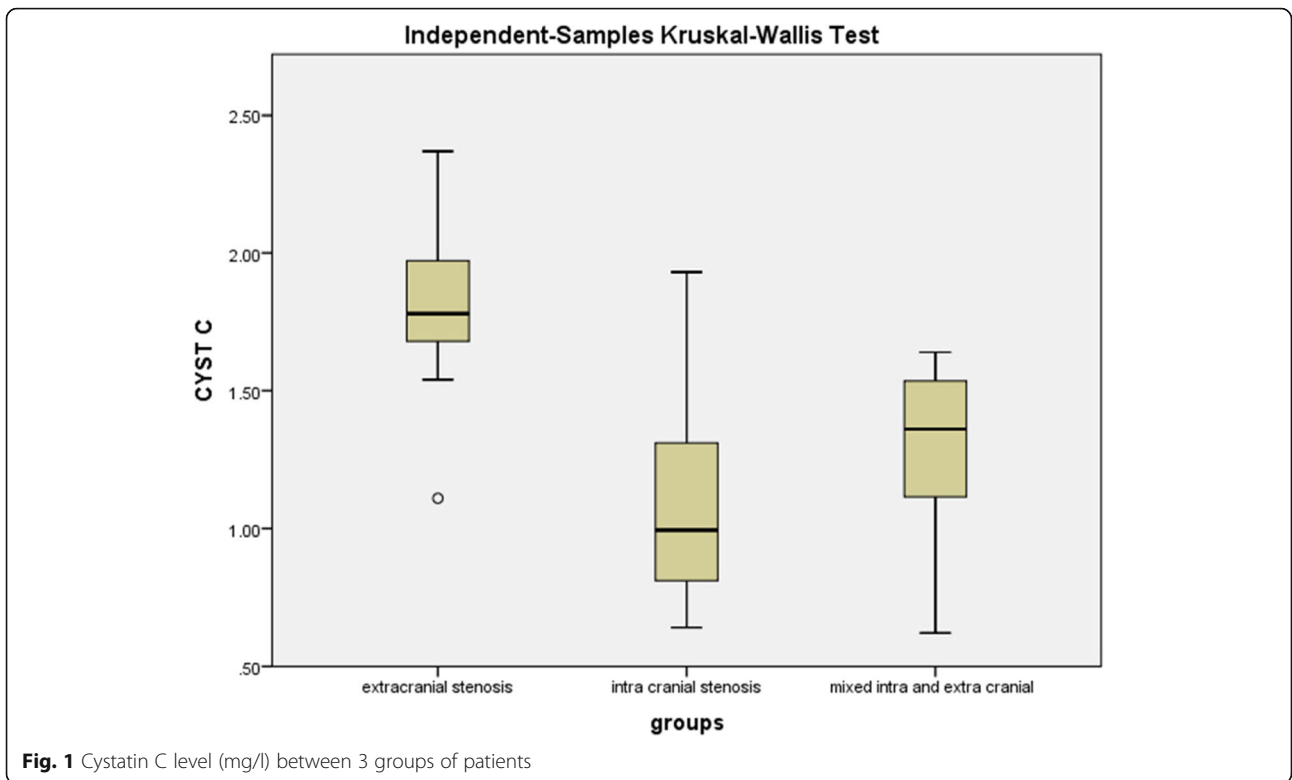
### Comparative analysis

Stroke patient (group A) had a significant higher level of CysC than healthy persons (group B) (*P* value = < 0.001). Moreover, patients with increased IMT had a higher level of CysC in comparison with patients with normal IMT in group A and all subjects in group B (*P* value = 0.003). The highest level of CysC was in patients with extracranial stenosis with statistical significance *P* value = 0.001, and it was high in patients with extracranial stenosis compared to patients with combined intra- and extracranial stenosis with *P* value = 0.015 (Fig. 1). Patients with > 50% stenosis have high level of CysC compared to patients with < 50% stenosis with statistical significance *P* value = 0.004 (Fig. 2). Among 19 patients with > 50% stenosis, CysC level was highest in extracranial stenosis (*P* value = 0.006). Intracranial stenosis > 50% had a higher level of CysC than to patients with intracranial stenosis < 50% with (*P* value = 0.03) (Fig. 3). The higher the patient score on NIHSS, the higher the level of CysC with (*P* value = < 0.001) (Table 1). No statistically significant difference was found between the level of CysC and size of infarction (*P* value = 0.403) (Table 2). Also, no statistically significant difference was found between CysC level and age or gender of patients, the presence or absence of hypertension, diabetes, and smoking. Regarding lipid profile and other laboratory findings in comparison to CysC level, no statistical significance was detected.

### Discussion

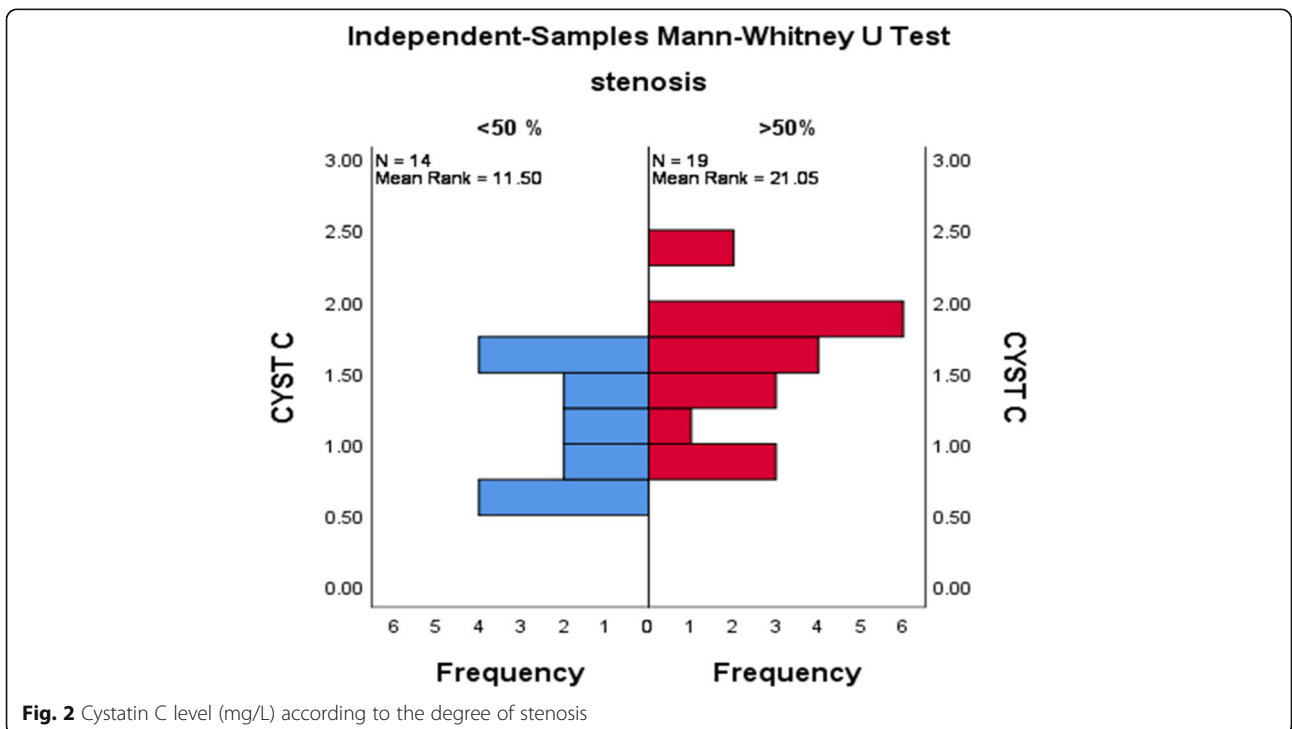
In this study, we compared the serum level of CysC between ischemic stroke patients and healthy individuals, and we observed that CysC level was higher in patients within the first week of stroke onset than in control subjects. These findings interpreted in the context that CysC as an inhibitor of cysteine protease and high level of CysC affects the process of vascular remodeling by breaking the balance of proteolytic and anti-proteolytic activities. Therefore, CysC acts as a good biomarker for acute ischemic stroke. Going with our result, Xu and colleagues [6] noticed that elevated CysC levels were strongly associated with stroke. Yang and colleagues [7] have similar results and hypothesized that Cyst C plays an important role in the pathogenesis of atherosclerosis.

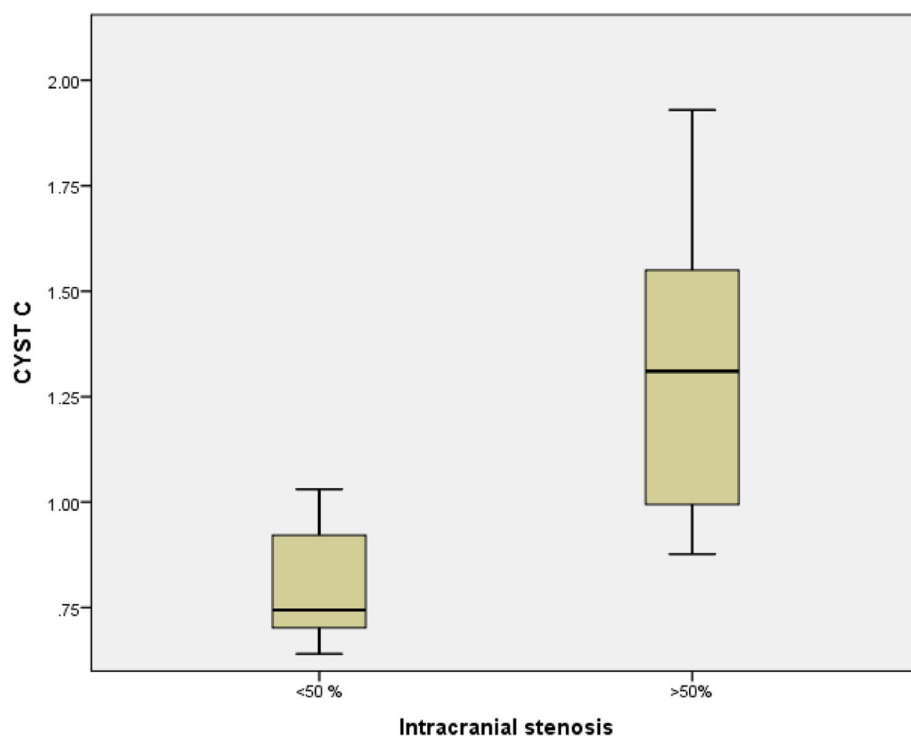
We should address that our study is limited by the small sample size which makes sensitivity 66% and specificity 100%.



In the present study, we found that there is no relation between the size of infarction and level of CysC, and we explain that serum levels of CysC were not affected by the size of infarction. First, because of the small number

of participants with total MCA and combined MCA PCA territory infarction in comparison to patients with lacunar and branch MCA infarction. Second, that size of actual infarction may be smaller in brain image, and it





**Fig. 3** Cystatin C level (mg/l) in patients with intracranial stenosis according to the degree of stenosis

**Table 1** Level of CysC according to groups, IMT, site, and degree of stenosis

CysC Mg/L						P value
Group A (stroke patients)			Group B (control)			
Mean	SD	Median	Mean	SD	Median	
1.40	0.47	1.49	0.82	0.14	0.62	< 0.001
Normal IMT			Increased IMT			0.003
Mean	SD	Median	Mean	SD	Median	
1.00	0.39	0.92	1.55	0.42	1.56	
< 50% stenosis			> 50% stenosis			0.004
Mean	SD	Median	Mean	SD	Median	
1.11	0.39	1.07	1.61	0.42	1.55	
< 50% extracranial stenosis (2 patients)			> 50% extracranial stenosis (9 patients)			0.073
Mean	SD	Median	Mean	SD	Median	
1.37	0.37	1.37	1.92	0.27	1.88	
< 50% intracranial stenosis (5 patients)			> 50% intracranial stenosis (6 patients)			0.03
Mean	SD	Median	Mean	SD	Median	
0.81	0.16	0.74	1.33	0.39	1.31	
< 50% combined extra and intracranial stenosis (7 patients)			> 50% combined extra and intracranial stenosis (4 patients)			0.927
Mean	SD	Median	Mean	SD	Median	
1.26	0.41	1.36	1.31	0.25	1.38	

CysC cystatin C; IMT intima media thickness; SD standard deviation

**Table 2** Level of CysC according to size of infarction

CysC Mg/L	Size of infarction				P value
	Lacunar (3 patients)	Branch MCA (21 patients)	Total MCA (8 patients)	Total MCA and PCA (1 patients)	
Mean	1.27	1.41	1.52	0.64	0.403
SD	0.45	0.46	0.49	–	
Median	1.11	1.49	1.56	0.64	

CysC cystatin C, MCA middle cerebral artery, PCA posterior cerebral artery, SD standard deviation

appears large due to the presence of penumbra and vesogenic edema. On the other hand, Xiao and colleagues found that larger cerebral infarction size is associated with higher serum CysC. Therefore, CysC may be a predictor for the severity of ischemia and cell damage [8].

In the current study, a positive correlation was found between CysC level and NIHSS scores with statistical significance, so we concluded that CysC is a prognostic tool for stroke severity as the higher the CysC level, the severer the stroke. Zhiqiang and colleagues [6] show a positive correlation between CysC and NIHSS. While Zeng and colleagues [9] observed that correlation between CysC levels and NIHSS was not significant, that was conversely, to our results due to our selection criteria as our patients were in the first week of stroke.

As regards CysC predictive value, in the present study, increased IMT had a higher level of CysC with positive statistical significance. Matching with our result, Zhu and colleagues [10] noticed that CysC was strongly associated with carotid thickening and plaque. Moreover, Kaneko and colleagues [11] noticed that CysC is strongly and independently associated with arterial wall elasticity, which reflects the degree of subclinical atherosclerosis. On the contrary, Bui and colleagues [12] found no association between CysC with internal and common carotid IMT and such differences are due to their participants that were four different self-reported ethnic groups (white, African American, Hispanic, and Chinese).

In our study, when we compared the level of CysC between patients with extracranial, transcranial, and combined stenosis, we noticed that the highest level of CysC present in patients with extracranial stenosis with statistical significance that can be explained by that CysC contributes to the process of carotid atherosclerosis. Extracranial arteries are elastic arteries while intracranial arteries are muscle arteries, and atherosclerosis and vascular calcification occur in extracranial vessels while oxidative stress which induces endothelial dysfunction occurs in intracranial vessels. Moreover, patients with stenosis > 50% had higher levels of CysC compared to patients with stenosis < 50% with positive statistical

significance. Extracranial stenosis > 50% had the highest level of CysC with positive statistical significance. Xu and colleagues [13] noticed that serum CysC was highly associated with large cerebral artery stenosis but not the location of large cerebral artery stenosis whether extracranial or intracranial. Going with our result, Umemura and colleagues [14] suggested that higher levels of CysC were independently associated with symptomatic extracranial ICA stenosis, but not with intracranial ICA/MCA stenosis in patients with non-cardioembolic stroke.

Peliseket and colleagues [15] also suggested that an imbalance between cysteine proteinase and CysC could impact changes in vascular structure and stenotic lesions. While Huang and colleagues [16] observed serum CysC is high in stroke patients but were not correlated with the presence of intracranial arterial stenosis.

In the present study, patients' age and gender had no correlation with CysC level, and our finding implies that CysC was included in the process of carotid atherosclerosis via chronic inflammation and not related to demographic risk factors. Matching with our result, Yang and colleagues [7] observed no statistical significance between CysC and age or gender of stroke patients. Although Kobayashi and colleagues [17] observed a higher level of CysC in males.

In the current study, no significant correlation was found between CysC level and hypertension, diabetes, or smoking. So CysC is strongly and independently associated with arterial wall atherosclerosis. Going with our finding, Xiao and colleagues [8] and Huang and colleagues [16] found that serum CysC concentrations in stroke patients were not correlated with hypertension or diabetes. On the other hand, Yang and colleagues [7] observed a significant association between CysC level and hypertension and between CysC and diabetes. Kobayashi and colleagues [17] also noticed statistical significance between CysC level and hypertension, DM, and smoking, taking into consideration that there were few participants with severe kidney disease in these studies.

In our study, no statistical significance was found in comparing cystatin c level and lipid profile. Matching with our study result, Xiao and colleagues [8] and Huang and colleagues [16] found that CysC levels were not correlated with serum total cholesterol, high-density lipoprotein, and low-density lipoprotein levels. While on the contrary, Xu and colleagues [6] observed a significant correlation with serum CysC levels and serum triglycerides and cholesterol.

In the present study, no statistical significance was found between CysC level and electrolyte. Going with our finding, Gharaibeh and colleagues [18] found that serum electrolyte levels did not show any significant changes in cases compared to controls.

## Conclusion

From all previous results, it could be concluded that serum cystatin C can be used as a marker for acute atherosclerotic ischemic stroke and good indicator for stroke severity. Higher levels of CysC closely associated with extracranial stenosis than with transcranial stenosis. So, CysC is a good predictor, diagnostic, and prognostic biomarker in acute stroke.

## Abbreviations

AIS: Acute ischemic stroke; CKD: Chronic kidney disease; CT: Computerized tomography; CysC: Cystatin C; DM: Diabetes mellitus; eGFR: Estimated glomerular filtration ratio; ICA/MCA: Internal carotid artery/middle cerebral artery; IMT: Intima-media thickness; LAS: Large artery strokes; MCA: Middle cerebral artery; NIHSS: National Institutes of Health Stroke Scale; PCA: Posterior cerebral artery; TG: Triglycerides; US: Ultrasonography

## Acknowledgements

Not applicable.

## Authors' contributions

MAZ participated in the concept and the study design, applying the inclusion and exclusion criteria and drafting the manuscript. SAS participated in the concept and design of the study and is responsible for the laboratory handling, preparation, and analysis of the blood sample. NSW participated in the concept and design of the study, is responsible for the coordination and recruitment of participants, and helped in the clinical examination and data analysis. AMA participated in the design of the study and analysis of data, helped in the clinical examination, helped in applying the inclusion and exclusion criteria, and performed the statistical analysis. All authors read and approved the final manuscript.

## Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

## Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available due to the current Cairo University regulations and Egyptian legislation but are available from the corresponding author on reasonable request and after institutional approval.

## Ethics approval and consent to participate

Informed written consent was taken from each patient. All data obtained from every patient were confidential and were not used outside the study. The patients have the rights to withdraw from the study at any time without giving any reason. All the cost of the investigations was afforded by the researcher.

Our study was approved by the ethical committee of the Department of Neurology, Faculty of Medicine, Cairo University, on 11th of November 2017, but Cairo University does not provide the approval reference number.

## Consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

## Author details

<sup>1</sup>Department of Neurology, Faculty of Medicine, Cairo University, Al Orman Giza Governorate 12613, Giza, Egypt. <sup>2</sup>Department of Chemical and Clinical Pathology, Faculty of Medicine, Cairo University, Al Orman Giza Governorate 12613, Giza, Egypt.

Received: 28 January 2019 Accepted: 3 July 2019

Published online: 15 July 2019

## References

1. Salgado JV, Souza FL, Salgado BJ. How to understand the association between cystatin C levels and cardiovascular disease: Imbalance, counterbalance, or consequence? *J Cardiol*. 2013;62(6):331–5.
2. Tahmasebpour HR, Buckley AR, Cooperberg PL. Fix CH Sonographic examination of the carotid arteries. *Radiographics*. 2005;25:1561–75.
3. Chan YH. Biostatistics 102: Quantitative data—parametric and non-parametric tests. *Singapore Med J*. 2003a;44(8):391–6.
4. Chan YH. Biostatistics 103: Qualitative data-tests of independence. *Singapore Med J*. 2003b;44(10):498–503.
5. Chan YH. Biostatistics 104: Correlational analysis. *Singapore Med J*. 2003c;44(12):614–9.
6. Xu Z, Leng C, Yang B, Wang H, Sun J, Liu Z, et al. Serum cystatin C is associated with large cerebral artery stenosis in acute ischemic stroke. *Oncotarget*. 2017;8(40):67181–8.
7. Yang B, Zhu J, Miao Z, Zhou B, Ge W, Zhao H, et al. Cystatin C is an independent risk factor and therapeutic target for acute ischemic stroke. *Neurotox Res*. 2015;28:1–7.
8. Xiao D, Liu H, Zhang H, Luo Y. Impact of cystatin C levels on infarct size and hemorrhage volume in acute cerebral stroke. *J Neurol*. 2012;259(10):2053–9.
9. ZengQ LK, YaoM WL. Significant correlation between cystatin C, cerebral infarction, and potential biomarker for increased risk of stroke. *CurrNeurovasc Res*. 2015;12(1):40–6.
10. Zhu Y, Zhang HP, Wang YC, Ren TT, Li J, Xu ML, et al. Serum cystatin C level is associated with carotid intima-media thickening and plaque. *Scand J Clin Lab Invest*. 2015;75:265–72.
11. Kaneko R, Sawada S, Tokita A, Honkura R, Tamura N, Kodama S, et al. Serum cystatin C level is associated with carotid arterial wall elasticity in subjects with type 2 diabetes mellitus: a potential marker of early-stage atherosclerosis. *Diabetes Res Clin Pract*. 2018;139:43–51.
12. Bui AL, Katz R, Kestenbaum B, de Boer IH, Fried LF, Polak JF, et al. Cystatin C and carotid intima-media thickness in asymptomatic adults: the Multi-Ethnic Study of Atherosclerosis (MESA). *Am J Kidney Dis*. 2009;53(3):389–98.
13. Xu Y, Ding Y, Li X, Wu X. Cystatin C is a disease-associated protein subject to multiple regulation. *Immunol Cell Biol*. 2015;93(5):442–51.
14. Umemura T, Kawamura T, Mashita S, Kameyama T, Sobue G. Higher levels of cystatin C are associated with extracranial carotid artery steno-occlusive disease in patients with noncardioembolic ischemic stroke. *Cerebrovasc Dis Extra*. 2016;6:1–11.
15. Pelisek J, Hahntow IN, Eckstein HH, Ockert S, Reeps C, Heider P, et al. Impact of chronic kidney disease on carotid plaque vulnerability. *J Vasc Surg*. 2011 Dec;54(6):1643–9.
16. Huang GX, Ji XM, Ding YC, Huang HY. Association between serum cystatin C levels and the severity or potential risk factors of acute ischemic stroke. *Neurol Res*. 2016;38(6):518–23.
17. Kobayashi T, Yokokawa H, Fujibayashi K, Hanui T, Hisaoka T, Fukuda H, et al. Association between high cystatin C levels and carotid atherosclerosis. *World J Cardiol*. 2017;9(2):174–81.
18. Gharaibeh KA, Hamadah AM, El-Zoghby ZM, Lieske JC, Larson TS, Leung N. Cystatin C predicts renal recovery earlier than creatinine among patients with acute kidney injury. *Kidney Int Rep*. 2017;3(2):337–42.

## Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.