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The diagnostic utility of unenhanced computed tomography of the brain and D-dimer levels in acute cerebral venous sinus thrombosis: A quantitative study

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ABSTRACT

Objectives: (1) To calculate the sensitivity and specificity of the Hounsfield Unit (HU), the HU to hematocrit (H:H) ratio, and the D-dimer level in the diagnosis of acute CVST. (2) To assess the D-dimer level's linear relationship with the HU and the H:H ratio.

Materials and Methods: A single-center retrospective case-control study was conducted from 2005 to 2020. The inclusion criteria for the thrombosed and control groups were specified. A region of interest (ROI) was plotted on the respective sinuses to calculate the HU. The H:H ratio was calculated by dividing the HU value by the hematocrit value. The receiver operating characteristic curve was used to calculate the sensitivity and specificity of the HU and the H:H ratio at different cutoff values. The Pearson correlation was used to assess the linear relationship between the D-dimer level and the HU and H:H ratio.

Results: There were 19 patients in the thrombosed group and 28 patients in the control group. There were significant differences in the mean HU (71 ± 6.3 vs. 45 ± 4.8, $P < 0.001$) and the mean H:H ratio (2.11 ± 0.38 vs. 1.46 ± 0.63, $P < 0.001$). An optimal HU value of 56 yielded 100% sensitivity and specificity. An H:H value of 1.48 yielded a sensitivity of 100% and a specificity of 65%, an H:H ratio of 1.77 demonstrated a sensitivity of 85% and a specificity of 90%, and an H:H ratio of 1.88 yielded a sensitivity of 79% and a specificity of 93%. D-dimer levels had a 95% and 71% sensitivity and specificity, respectively. There was a significant moderately positive linear correlation between the D-dimer level and the HU ($r = 0.52$, $P < 0.001$) and the H:H ratio ($r = 0.61$, $P < 0.001$).

Conclusion: Unenhanced CT of the brain can be a valuable objective diagnostic tool for acute CVST diagnosis. Hounsfield blood density and its normalized ratio with hematocrit are positively correlated with D-dimer levels, which may indicate active blood coagulation in a cerebral venous sinus.

Keywords: Acute cerebral venous sinus thrombosis, Hounsfield unit, Hounsfield:Hematocrit ratio, D-dimer

INTRODUCTION

Cerebral venous sinus thrombosis (CVST) is a rare condition with an estimated annual incidence of 2–7 per million.[1] It is a serious entity, and failure to recognize and treat it can lead to devastating consequences like strokes, hemorrhages, and even death.[2] It is a clinically challenging diagnosis as it typically presents with nonspecific neurological manifestations, most commonly headaches, altered mental status, focal neurological deficits, and seizures.[1] The most common predisposing risk factors for cerebral sinus thrombosis are hypercoagulable states like malignancy, thrombophilia conditions, and high estrogenic states such as during pregnancy and when using oral contraceptives.[3] Other predisposing factors include local infections, skull fractures, and brain surgeries.[3] However, CVST can be idiopathic in 10–20% of cases.[3]

Unenhanced CT of the brain is an initial and cost-effective modality to investigate intracranial pathology, especially in an emergency setting. It can raise suspicion for acute CVST by subjective visualization of increased sinus attenuation. Many studies have proposed useful objective diagnostic methods for acute CVST in unenhanced CT of the brain, such as the Hounsfield Unit (HU) density of the sinus and the more reliable normalized ratio of the HU to hematocrit (H:H).[4-8]

The D-dimer level is an inexpensive blood test used to evaluate multiple nonocclusive diseases such as pulmonary embolism and deep venous thrombosis. A study review demonstrated its valuable contribution in predicting CVST when combined with other diagnostic parameters.[9]

Our study aimed to investigate the objective measures of the unenhanced CT of the brain, namely the HU and the H:H ratio for the diagnosis of acute CVST in our setting. A second novel aim was to assess the linear relationship between the D-dimer level and the HU and the H:H ratio.

MATERIALS AND METHODS

This single-center retrospective case-control study was conducted at King Faisal Specialist Hospital & Research Centre (KFSH & RC), Riyadh, Saudi Arabia. Ethical approval was obtained from the hospital's Research Advisory Committee (Approval number: 2211025). No informed consent was obtained from the patients due to the anonymized and retrospective nature of our study. The inclusion criteria for the thrombosed and control groups were (1) The patient presented with an acute neurological manifestation (<3 days), (2) Initial unenhanced CT of the brain was performed upon presentation, (3) A confirmatory CT brain venogram (CTV) or contrast-enhanced MR venogram (CE-MRV) was performed within 24 hours of the unenhanced CT of the brain, (4) The D-dimer level was measured within 24 hours of the unenhanced CT of the brain, and (5) Hematocrit level was measured within 24 hours of unenhanced CT of the brain. Patients were excluded from the study if they did not meet one or more of the above-mentioned five inclusion criteria in both the thrombosed and control groups. Participants were also

Figure 1a: A 39-year-old woman with antiphospholipid syndrome who presented with a headache. (a) A non enhanced CT image in this control patient shows two ROIs plotted on the sigmoid sinuses at the jugular fossa.

Figure 1b: A 39-year-old woman with antiphospholipid syndrome who presented with a headache. (b) A non enhanced CR image in the same control patient shows the ROI plotted on the posterior superior sagittal sinus. The average mean HU value of the three ROIs is 42.33. Contrast-enhanced CT brain (not shown) shows no evidence of venous thrombosis.

excluded if there were technical issues in the unenhanced CT of the brain, such as motion artifacts or beam hardening artifacts, precluding accurate measurements.

Initially, all CTV and MRV examinations were extracted from the Picture Archiving and Communication System (PACS) and patient charts for 2005–2020. Subsequently, the patients were included in the thrombosed or control groups based on the fulfillment of the inclusion criteria.

All patients were imaged using 64-slice multidetector scanners utilizing 120 kVp, 300 mAs, and 5 mm slice thickness. The best-fit size region of interest (ROI) was plotted over the sinus to calculate the HU, avoiding the adjacent dura, bone, and adjacent brain parenchyma to avoid volume averaging. Examples of the measurements are shown in [Figures 1 and 2].

In the thrombosed group, the HU was recorded from the thrombosed sinus/vein. If there were multiple thrombosed veins/sinuses, the ROIs were plotted on each of them, and the average HU was computed. The normalized HU with respect to hematocrit (H:H ratio) was calculated by dividing the single (in the case of a single thrombosed sinus) or the average thrombosed HU value (in case of multiple thrombosed sinuses) by the hematocrit value (g/dL). The HU of the control group was determined by computing the average HU value of the posterior superior sagittal sinus (SSS) and bilateral sigmoid sinus (SS) at the jugular foramen. Then, the H:H ratio was calculated by dividing the average HU over the hematocrit value (g/dL). HU values were recorded by a neuroradiology consultant and a final-year radiology registrar. The average values of the recordings were employed for subsequent analysis. D-dimer levels were interpreted as either elevated (≥500 µg/L) or normal (<500 µg/L). Enzyme-linked immunosorbent assays (ELISA) were used for D-dimer testing.

Statistical analysis was performed using the Statistical Package for Social Science version 25 (SPSS Inc., Chicago, IL) software. The intraclass correlation coefficient (ICC) was used to assess the inter-rater reliability of the HU readings. All continuous variables were tested for normality using the Shapiro-Wilk test and were normally distributed, except for H:H ratio. The mean values of age, hematocrit, HU, and H:H ratio were compared between the thrombosed and control groups using the independent sample *t*-test or Mann-Whitney U test. The Chi-square test was used to compare D-dimer and gender between the thrombosed and control groups. Receiver-operating characteristics (ROC) curves were generated to determine the sensitivity and specificity of the HU and the H:H ratio at different cutoff values. The Pearson correlation was used to assess the linear relationship of the D-dimer levels with the HU and the H:H ratio. A *P*-value of <0.05 and a 95% confidence interval was used to indicate the statistical significance and precision of the results, respectively.

RESULTS

Over the 15 years study period, there were 48 patients diagnosed with CVST; among them, there were 19 patients who fulfilled the inclusion criteria and were included in the thrombosed group. Twenty-eight control patients satisfied the inclusion criteria. The location sites of thrombosis in the thrombosed group are shown in [Table 1].

The ICC for inter-rater reliability of HU measurements was 0.92 (0.89–0.94), indicating a good to excellent result. The HU and H:H ratio values for the control and the thrombosed groups are shown in [Figures 3 and 4]. There were no statistical differences between the thrombosed and control groups in age, gender, and hematocrit level (P = 0.53, 0.31, and 0.37, respectively) [Table 2]. There were statistically significant differences between the thrombosed and control groups in the HU, H:H ratio, and D-dimer levels (all P < 0.001, Table 2). The mean HU value was 70.8 in the thrombosed group and 45.1 in the control group. The mean H:H ratio was 2.11 in the thrombosed group and 1.46 in the control group.

The ROC curve was generated for the HU yielding an area under the curve of 1 [Figure 5]; there was no overlap of the HU values between the thrombosed and control groups.

An HU of 56 was selected as the optimal value, as it had the lowest HU value yielding 100% sensitivity and specificity. The ROC curve was generated for the H:H ratio values yielding

Table 1: Location of thrombus in the thrombosed group.

Patient	Age	Gender	Thrombosed sites
1	24	Female	SSS
2	15	Male	Right TS and Right SS
3	19	Female	SSS
4	7	Female	Right TS and Right SS
5	50	Female	Left TS and Left SS and Left JB
6	68	Female	Right TS and Right SS and Right JB
7	36	Male	SSS and Right TS
8	51	Female	Right TS and Right SS and Right JB
9	12	Female	SSS and Bilateral SS, TS, and JB
10	9	Male	SSS
11	67	Female	SSS and Left TS
12	42	Female	Right SS
13	5	Male	SSS, Right TS
14	6	Female	SSS
15	29	Female	SSS and Left TS and Left SS and Left JB
16	18	Male	Left TS and Left SS and Left JB
17	37	Male	SSS and Right TS
18	52	Male	Right parietal cortical vein
19	29	Male	Right SS

SSS: Superior Sagittal Sinus, TS: Transverse Sinus, SS: Sigmoid Sinus, JB: Jugular Bulb

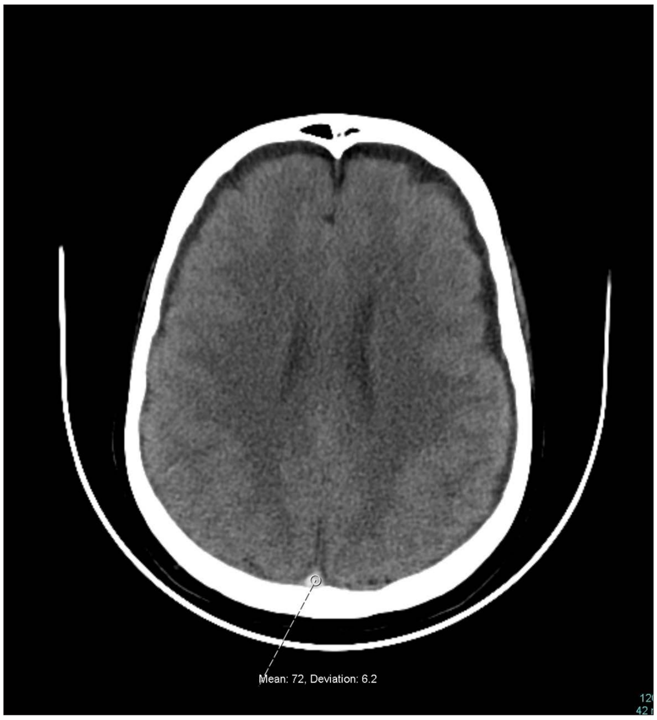

Figure 2a: A 37-year-old woman with acute lymphoblastic leukemia who presented with headache and blurry vision. (a) Non-enhanced axial CT image of the brain shows increased attenuation of the superior sagittal sinus (HU = 72).

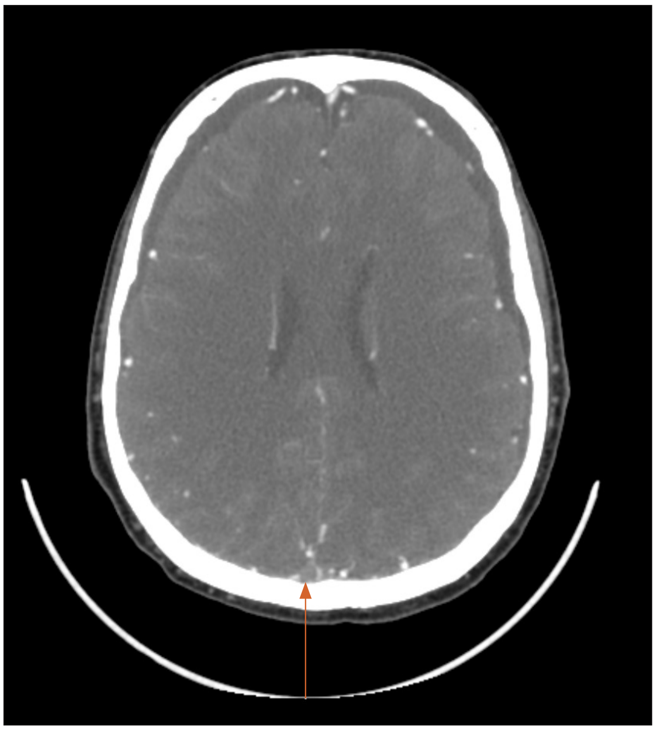

Figure 2b: A 37-year-old woman with acute lymphoblastic leukemia who presented with headache and blurry vision. (b) Contrast-enhanced axial image at the same location shows a filling defect of the superior sagittal sinus consistent with thrombosis (arrow).

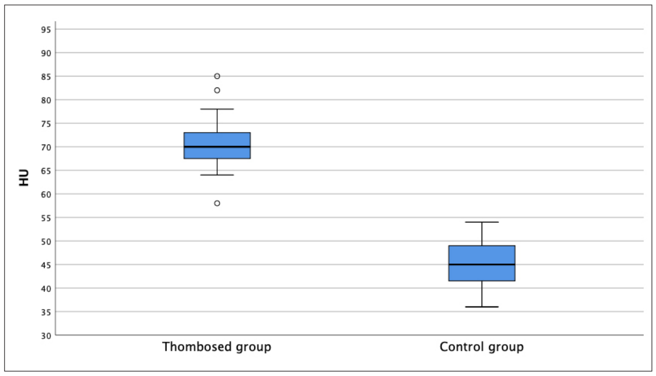

Figure 3: Box and whisker plot of the HU values in the thrombosed and control groups.

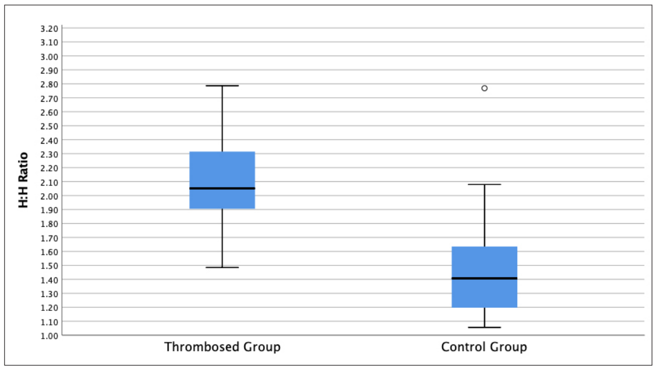

Figure 4: Box and whisker plot of the H:H ratio values in the thrombosed and control groups.

an area under the curve of 0.908 [Figure 5]. An H:H value of 1.48 yielded a sensitivity of 100% and a specificity of 65%, an H:H ratio of 1.77 demonstrated a sensitivity of 85% and specificity of 90%, and an H:H ratio of 1.88 yielded a sensitivity of 79% and specificity of 93%. The study showed that positive D-dimer levels had 95% and 71% sensitivity and specificity, respectively, for acute CVST diagnosis. There was only one patient in the thrombosed group with a negative D-dimer value, with the level being in the upper limit of normal (465 µg/L).

Pearson's correlation coefficient was used to assess the relationship between the D-dimer level with the HU and H:H ratio for the combined thrombosed and control groups. Figure 6 showed a statistically significant moderate positive linear correlation ($r = 0.52$) between the D-dimer level and the HU ($P < 0.001$) and Figure 7 shows a statistically significant moderate positive linear correlation ($r = 0.61$) between the D-dimer level and the H:H ratio ($P < 0.001$). Hence a slightly higher correlation coefficient was observed between the D-dimer and the H:H ratio than with the HU.

DISCUSSION

Unenhanced CT of the brain is a cost-effective initial modality for acute neurological manifestations.[3] The classic imaging feature

Table 2: Study characteristics of the thrombosed group and control group.

Characteristics	Thrombosed group (N=19)	Control group (N=28)	*P*
Mean age*, years	30.3 (20.3)	27.2 (13.3)	0.529
Gender: Male N (%)	8 (42%)	16 (57%)	0.312
Gender: Female N (%)	11 (58%)	12 (43%)	
Hematocrit*, g/dL	34.3 (8.8)	32.2 (7.2)	0.369
Hounsfield unit*	70.8 (6.3)	45.1 (4.8)	<0.001
H:H ratio*	2.11 (0.38)	1.46 (0.36)	<0.001
D-dimer: Positive N (%)	18 (95%)	8 (29%)	<0.001
D-dimer: Negative N (%)	1 (5%)	20 (71%)	

*Data are reported as mean (standard deviation). *P*-values were calculated by independent sample *t*-test, Mann-Whitney U test or Chi-square test.

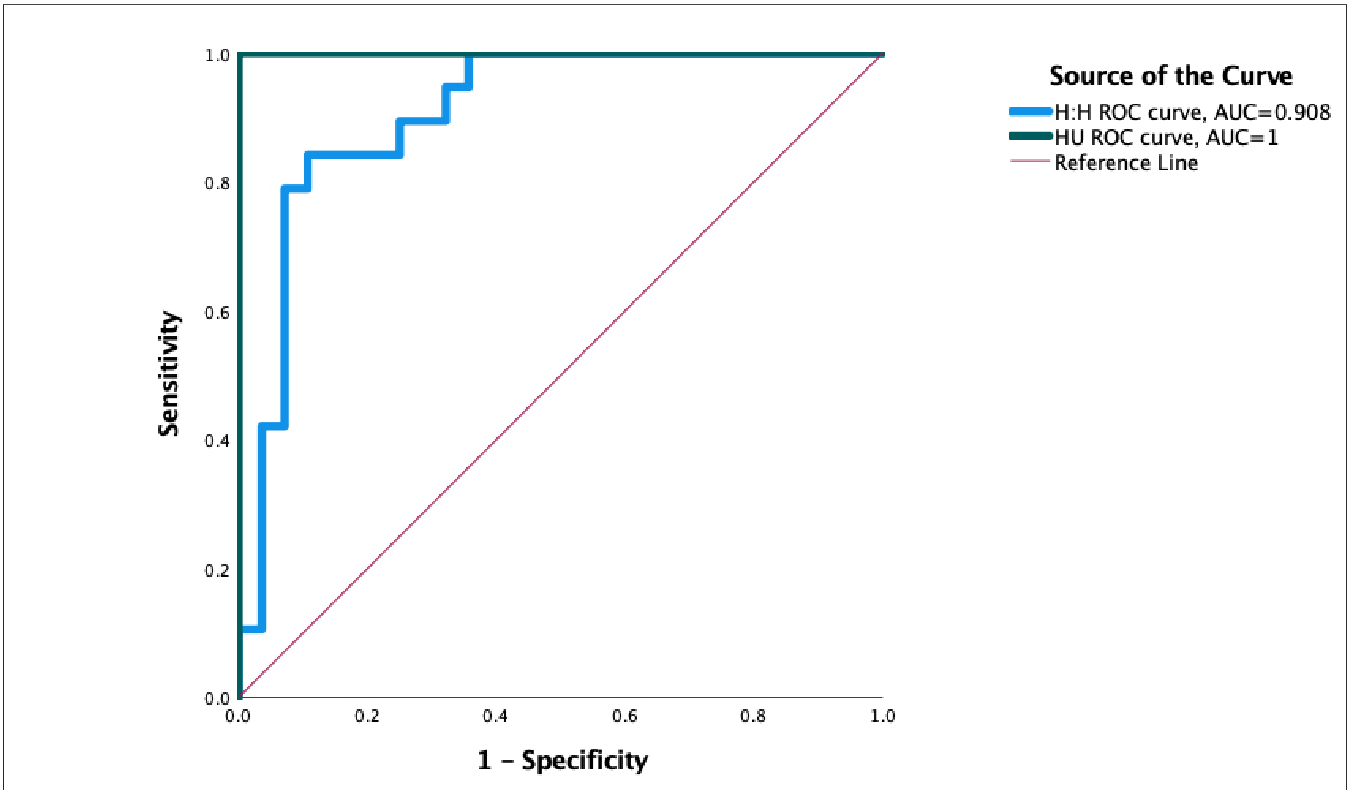

Figure 5: The ROC curves of the HU and the H:H ratio.

for acute CVST in an unenhanced CT of the brain is the subjective hyperattenuating vessel, with a sensitivity reaching up to 73%.[10,11] Hyperattenuation is related to the clot retraction caused by decreased water content and the increased concentration of proteins and red blood cells.[3,10] Hyperdense vessels usually become isodose to the normal vessel after 2 weeks.[3] Other important indirect signs depicted in the unenhanced CT of the brain are brain parenchymal edema and hemorrhages, which may or may not occur in a venous territory.[3,10] The edema can occur in two forms: cytotoxic and vasogenic. These parenchymal changes are more likely in locations where collaterals are insufficient, such as cortical vein involvement.[10] Edema can be reversible if no bleeding has developed, adequate collaterals are present, and there is no cellular death.[10] The involved sinuses from most to least frequent are the SSS, transverse sinus, SS, deep veins, and the cortical vein.[10]

The definitive diagnosis of CVST can be made by either CT venography or enhanced/unenhanced MRI of the brain.[3,12] Unenhanced MR techniques are the two-dimensional time of flight and the phase-contrast MR.[3] Contrast-enhanced MR techniques are preferred and more accurate than

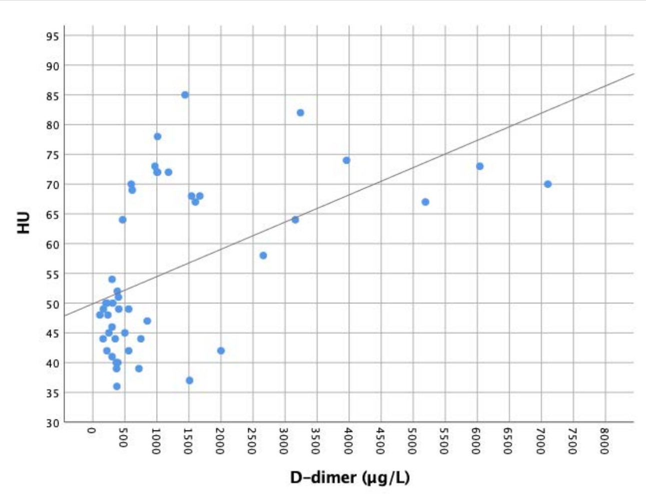

Figure 6: Pearson's correlation between the HU and the D-dimer ($r = 0.52$, $p < 0.001$).

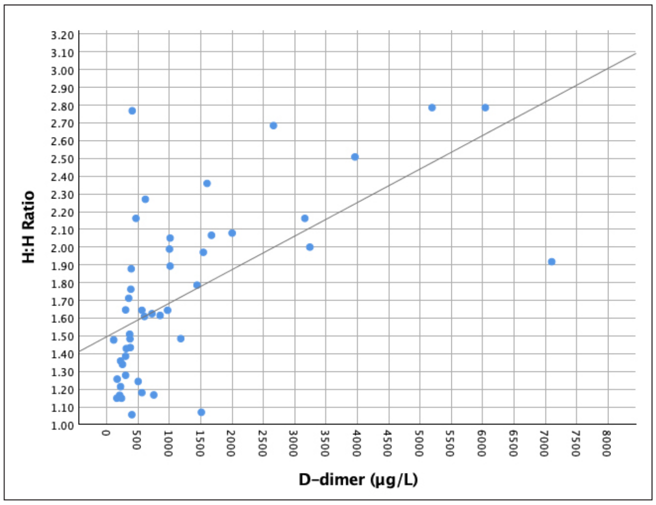

Figure 7: Pearson's correlation between the H:H ratio and D-dimer ($r = 0.61$, $P < 0.001$).

unenhanced techniques.[3,12] Both contrast-enhanced MR and CT venography are adequate for the diagnosis, and the choice between them depends on patient factors, availability, local preference, and experience.[12] In our study, the confirmatory study was carried out using either CT venography or contrast-enhanced MR venography.

Many recent studies have shown promising results of unenhanced CT of the brain utilizing objective quantitative measures to diagnose acute CVST.[4,6-8,13] These methods are easily acquired and may allow a more confident diagnosis of vessel hyperattenuation than subjective evaluation. The two most commonly suggested and used objective measures are the HU and the normalized H:H ratio. There is evidence of a positive moderate linear correlation between the hematocrit level and vessel attenuation in HU ($r = 0.5$, $P < 0.001$).[7] However, the blood vessel can have a low density in states where there are low hematocrit levels, such as in anemia. The opposite is true in conditions with high hematocrit levels such as in young children, dehydration, and polycythemia, which can be sources of false-positive interpretation.[14,15] Thus, the H:H ratio is postulated to be more reliable where there are abnormal levels of hematocrit and more useful when combined with HU.[4,6-8]

In our study, there was a significant difference in the mean HU values between the thrombosed group and the control group (71 ± 6.3 vs. 45 ± 4.8, $P < 0.001$), as well as a significant difference in the mean H:H ratio values between the two groups (2.11 ± 0.38 vs. 1.46 ± 0.63, $P < 0.001$). Previous studies have shown mean HU values ranging between 66 and 77 in the thrombosed group, and mean values ranging between 47 and 53 in the control group.[4,6-8,13] With the H:H ratio, these studies reported mean values ranging between 1.75 and 2.2 in the thrombosed group and mean values ranging between 1.33 and 1.44 in the control group.[4,6-8,13] Our mean HU and H:H ratio values are approximately within the previous studies' ranges. In our study, there was no overlap in the HU values between the thrombosed and control groups, with the suggested HU value of 58 yielding 100% sensitivity and specificity. However, there was an overlap in the H:H ratio values, and multiple cutoff values with different sensitivity and specificity are proposed (at 1.48: sensitivity = 100%, specificity = 65%; at 1.77: sensitivity = 85%, specificity = 90%; and at 1.88: sensitivity = 79%, specificity = 93%). There is slight variability in the literature regarding the suggested or optimum HU and H:H ratio values. Besachio *et al.*[8] suggested an HU value of >65 and an H:H ratio greater than 1.7 to identify the majority of CVST cases. Buyck *et al.*[6] showed very high sensitivity and specificity of the HU and H:H ratio using cutoff values of 62 and 1.52, respectively. Muns *et al.* studied the HU and H:H ratio in the pediatric population and proposed an optimum HU value of 57, yielding 100% sensitivity and specificity, and an H:H ratio value of 1.4, leading to 100% sensitivity and 74% specificity.[4]

While objective measures can provide a more confident and discriminative evaluation of CVST, it is essential to note that false-positive results might occur. These objective measures do not obviate the need for definitive diagnostic studies. In our study, one outlier control case with a high H:H ratio (2.77) was identified to have a very low hematocrit level (17.7 g/dL); however, it had an acceptable HU value (49) and a negative D-dimer value. Muns *et al.*[4] reported that a pediatric patient with a visually hyperdense transverse sinus had a measured HU of 70; however, the calculated H:H ratio was low (0.8) and was attributed to the elevated hematocrit value (70 g/dL). Both examples showed the importance of combining multiple objective parameters and not relying on a single measure to increase diagnostic accuracy and avoid false-positive results.

A study review by Smith *et al.*[9] concluded that normal D-dimer levels on their own cannot reliably exclude CVST; however, it has a potential role in predicting CVST when combined with other clinical parameters. Alons *et al.*[16] and Heldner *et al.*[17] defined a low-risk group for CVST and demonstrated a high negative predictive value of D-dimer in this group. The normal unenhanced CT of the brain was included as one of the criteria in the low-risk population.[16] D-dimer was shown to be elevated in an acute and subacute CVST and positively correlated with the number or extension of thrombosed sinuses involved.[18,19] Only one patient in the thrombosed group had a negative D-dimer value in our study, with the level being in the upper limit of normal (465 µg/L). We also found that the D-dimer level had a positive moderate linear correlation with the HU and the H:H ratio, with a slightly higher positive correlation with the H:H (r = 0.61) ratio compared with the HU (r = 0.52), which has not been reported in the literature. Both of these findings might provide an approximate indication for active blood coagulation in a cerebral venous sinus.

The sampling bias limits our study as many thrombosed and control cases were excluded if all the inclusion criteria were not satisfied. Another limitation is the small sample size, limiting the generalizability of the data; however, it is a function of this rare diagnosis.

CONCLUSION

Unenhanced CT of the brain can be used as an objective tool that allows a more confident and discriminative evaluation of acute CVST. Combining multiple diagnostic parameters is essential to increase diagnostic accuracy and avoid false-positive results. D-dimer levels are positively correlated with HU and H:H values, with a higher correlation with the H:H ratio than with HU.

Declaration of patient consent

Institutional Review Board (IRB) permission obtained for the study.

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Conflicts of interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

1. Stam J. Thrombosis of the cerebral veins and sinuses. N Engl J Med 2005;352:1791–8.
2. Ferro JM, Canhao P, Stam J, Bousser MG, Barinagarrementeria F, ISCVT Investigators. Prognosis of cerebral vein and dural sinus thrombosis: Results of the International Study on Cerebral Vein and Dural Sinus Thrombosis (ISCVT). Stroke 2004;35:664–70.
3. Canedo-Antelo M, Baleato-González S, Mosqueira AJ, Casas-Martínez J, Oleaga L, Vilanova JC, *et al.* Radiologic clues to cerebral venous thrombosis. Radiographics 2019; 39:1611–28.
4. de La Vega Muns G, Quencer R, Ezuddin NS, Saigal G. Utility of Hounsfield unit and hematocrit values in the diagnosis of acute venous sinus thrombosis in unenhanced brain CTs in the pediatric population. Pediatr Radiol 2019;49:234–9.
5. Shayganfar A, Azad R, Taki M. Are cerebral veins Hounsfield unit and H:H ratio calculating in unenhanced CT eligible to diagnosis of acute cerebral vein thrombosis? J Res Med Sci 2019;24:83. 1
6. Buyck PJ, De Keyzer F, Vanneste D, Wilms G, Thijs V, Demaerel P. CT density measurement and H:H ratio are useful in diagnosing acute cerebral venous sinus thrombosis. AJNR Am J Neuroradiol 2013;34:1568–72.
7. Black DF, Rad AE, Gray LA, Campeau NG, Kallmes DF. Cerebral venous sinus density on noncontrast CT correlates with hematocrit. AJNR Am J Neuroradiol 2011; 32:1354–7.
8. Besachio DA, Quigley 3rd EP, Shah LM, Salzman KL. Noncontrast computed tomographic Hounsfield unit evaluation of cerebral venous thrombosis: A quantitative evaluation. Neuroradiology 2013;55:941–5.
9. Smith E, Kumar V. BET 1: Does a normal D-dimer rule out cerebral venous sinus thrombosis (CVST)? Emerg Med J 2018;35:396–7.
10. Leach JL, Fortuna RB, Jones BV, Gaskill-Shipley MF. Imaging of cerebral venous thrombosis: Current techniques, spectrum of findings, and diagnostic pitfalls. Radiographics 2006;26 Suppl 1:S19–41; discussion S42–3.
11. Roland T, Jacobs J, Rappaport A, Vanheste R, Wilms G, Demaerel P. Unenhanced brain CT is useful to decide on further imaging in suspected venous sinus thrombosis. Clin Radiol 2010;65:34–9.
12. Van Dam LF, Van Walderveen MAA, Kroft LJM, Kruyt ND, Wermer MJH, van Osch MJP, *et al.* Current imaging modalities for diagnosing cerebral vein thrombosis—A critical review. Thromb Res 2020;189:132–9.
13. Alsafi A, Lakhani A, Carlton Jones L, Lobotesis K. Cerebral venous sinus thrombosis, a nonenhanced CT diagnosis? Radiol Res Pract 2015;2015:581437.
14. Healy JF, Nichols C. Polycythemia mimicking venous sinus thrombosis. AJNR Am J Neuroradiol 2002;23:1402–3.
15. Rodallec MH, Krainik A, Feydy A, Hélias A, Colombani JM, Jullès MC, *et al.* Cerebral venous thrombosis and multidetector CT angiography: Tips and tricks. Radiographics 2006;26 Suppl 1:S5–18; discussion S42–3.
16. Alons IM, Jellema K, Wermer MJ, Algra A. D-dimer for the exclusion of cerebral venous thrombosis: A meta-analysis of low risk patients with isolated headache. BMC Neurol 2015;15:118.
17. Heldner MR, Zuurbier SM, Li B, Martial RV, Meijers JCM, Zimmermann R, *et al.* Prediction of cerebral venous

thrombosis with a new clinical score and D-dimer levels. Neurology 2020;95:e898–909.
18. Wang HF, Pu CQ, Yin X, Tian CL, Chen T, Guo JH, *et al*. D-dimers (DD) in CVST. Int J Neurosci 2017;127:524–30.
19. Hiltunen S, Putaala J, Haapaniemi E, Salonen O, Tatlisumak T. D-dimer and clinicoradiologic features in cerebral venous thrombosis. J Neurol Sci 2013;327:12–4.

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