High platelet distribution width can independently predict testicular survival in testicular torsion among patients with steady-state sickle cell anemia

Essa A Adawi,¹ Mazen Ahmed Ghanem ,² Ahmed Mazen Ghanem,³ Manal A Safan,⁴ Mohamed G Elsayed,⁵ Mohammed A Aqeel⁶

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¹Urology, Jazan University, Jazan, Saudi Arabia

²Urology, Menoufia University Faculty of Medicine, Al Rehab City, New Cairo, Egypt

³GP, Cairo University Kasr Alainy Faculty of Medicine, Al Rehab City, New Cairo, Egypt

⁴Department of Medical Biochemistry and Molecular Biology, Menoufia University Faculty of Medicine, Al Rehab City, New Cairo, Egypt

⁵Pediatric Surgery, Jazan University, Jazan, Saudi Arabia

⁶Department of Anesthesia and Critical Care, Jazan University, Jazan, Saudi Arabia

Correspondence to

Dr Mazen Ahmed Ghanem; mazenganem99@yahoo.co.uk

ABSTRACT

Objective This study aimed to evaluate the predictive value of platelet volume indices (PVI), such as mean platelet volume (MPV), platelet distribution width (PDW), and plateletcrit (PCT), as prognostic indicators of testicular viability in torsion patients with steady-state sickle cell anemia (SCA) who underwent surgical exploration.

Methods Forty-eight patients with SCA with testicular torsion and 46 male control subjects were enrolled in the study. All patients underwent scrotal color Doppler ultrasonography before surgery, and PVI (MPV, PDW, and PCT) values were measured in all participants. Symptom duration and testicular volume were also recorded.

Results The testicular salvage rate in patients with SCA was 73% after surgery. Analyses showed that MPV, PDW, and PCT values were significantly higher in torsed SCA as compared with controls ($p < 0.05$). Orchiectomy in patients with SCA showed significantly higher MPV, PDW, and PCT values than the orchiopexy group ($p < 0.05$). The MPV values of orchiectomy patients showed a higher significant cut-off of ≥ 11.5 fL, which is higher than in torsed patients without SCA, as an indicator of testis survival. PDW also demonstrated a higher significant cut-off of ≥ 12.7 fL for detorsion outcomes in patients with SCA. Symptom duration of less than 7 hours was also significantly correlated with orchiopexy ($p \leq 0.001$). Univariate analysis showed that higher MPV, increased PDW, and symptom duration were indicative of the outcome of testicular detorsion in SCA. Multivariable analysis showed that increased PDW and symptom duration are prognostic parameters for testicular viability in SCA.

Conclusion Increased PDW and symptom duration can be used as parameters for predicting testicular detorsion outcomes in patients with steady-state SCA.

INTRODUCTION

Sickle cell anemia (SCA) is characterized by chronic hemolysis, inflammatory damage, and progressive vascular disease complications, such as acute chest syndrome, stroke, and priapism with acute scrotal pain.^{1,2} Scrotal pain in patients with SCA should always be

Key messages

What is already known about this subject?

- ▶ The risk of testicular atrophy and unnecessary surgery in patients with sickle cell anemia (SCA) with acute scrotal pain improved the need for novel diagnostic techniques.
- ▶ There is a relationship between testicular torsion and platelet activation that results in higher platelet volume indices (PVI).
- ▶ Considering the chronic hemolysis and inflammatory damage in SCA, we expect that generalized alterations in PVI will occur in SCA.

What are the new findings?

- ▶ Significantly higher levels of platelet distribution width (PDW) and mean platelet volume values were demonstrated among orchiectomy patients with SCA.
- ▶ An increased PDW in SCA with testicular torsion is associated with testicular salvage rate.
- ▶ The higher production of PVI by the activated platelet, induced by testicular torsion, is associated with testicular atrophy in SCA.
- ▶ These findings are mainly related to increased inflammatory and thrombotic activity, which is associated with increased platelet synthesis in SCA.

How might it impact on clinical practice in the foreseeable future?

- ▶ PDW can be used by physicians as a parameter for predicting a high-risk group of patients with SCA with acute scrotal pain, which is a candidate for emergency scrotal exploration.

treated as acute scrotal pathologies. Although majority of these pathologies are non-acute (eg, epididymo-orchitis), testicular torsion is a surgical emergency and should be diagnosed early and treated immediately to preserve a patient's testes.^{3,4} Testicular torsion can occur at any age, but usually occurs in young boys, with a bimodal incidence in the

pediatric population (during the first year of life and between the ages of 13 and 16 years).⁵ If treated within 6 hours of the presenting pain, there is a good chance of saving the affected testicle (90%–100% of the testicles will be saved). If treated within 6–12 hours, depending on the degree of the torsion, 20%–50% of the testicles will be saved. If treated within 12–24 hours, 0%–10% of the testicles will be saved.⁶ Nevertheless, the risk of testicular atrophy and unnecessary surgery in patients with SCA with acute scrotal pain suggests the need for novel diagnostic techniques.

Because the pathophysiology of sickle cell vascular complication is a microenvironmental inflammatory process, hematological inflammation parameters are needed for predicting the outcomes of testicular torsion in patients with SCA.^{7,8} The role of platelets in these interactions is well documented.^{9,10} Several studies confirmed that there is a relationship between testicular torsion, defined as an acute vascular disease, and platelet activation which results in higher mean platelet volume (MPV).^{11–13} Therefore, platelet volume indices (PVIs) can be introduced as potential markers of early diagnosis of testicular torsion.

Considering the chronic hemolysis and inflammatory nature of sickle cell disease, we expect that generalized alterations in hematological parameters will occur in SCA.¹⁴ To date, the utility of different PVIs, such as MPV, platelet distribution width (PDW), and plateletcrit (PCT), has not been analyzed as prognostic markers of testicular torsion in patients with SCA. The present study aimed to investigate the predictive value of PVIs, such as MPV, PDW, and PCT, in the outcome of testicular torsion in patients with SCA.

METHODS

Patients

One hundred and fifteen patients with steady-state SCA were referred to the emergency department with acute scrotal pain. From this group, only 48 patients diagnosed with testicular torsion were enrolled in the study and underwent surgical exploration.

Forty-six patients with clinically diagnosed healthy testicular torsion were also included as control subjects from patients admitted to the emergency department for testicular exploration, with a similar age between the two groups. The control subjects had no epididymo-orchitis, testicular tumor, history of scrotal trauma and surgery, or hematological diseases. All participants provided written informed consent before enrollment in the study.

Selection criteria

Inclusion criteria

Patients with a complaint of acute scrotal pain were initially included. Testicular torsion was diagnosed by scrotal color Doppler ultrasonography (CDUS) and was confirmed by surgical exploration. The included

participants with SCA were confirmed by qualitative and/or quantitative hemoglobin electrophoresis.

Exclusion criteria

Patients with SCA were included after excluding perinatal testicular torsion, other known testicular pathology (cryptorchidism, testicular tumor, and epididymo-orchitis), or cases associated with compromised vascular testicles. Excluded patients were also those with chronic hepatic, renal, or hematological (eg, myeloproliferative disorders and leukemia) diseases. Patients with previous overt stroke and acute pain crisis hospitalization within 1 year were also excluded. Any patient who underwent manual detorsion followed by elective testicular fixation at a later date was also excluded.

Clinical examination

All subjects underwent physical examination after their medical histories were taken. Clinical findings included scrotal characteristics (tenderness, erythema, and swelling), the affected side, high-riding testis, lower abdominal pain, fever ($>38.5^{\circ}\text{C}$), nausea/vomiting, and pyuria. Symptom duration was defined as the time between onset of acute symptoms and detorsion.

CDUS was performed in all patients at the time of admission as the mainstay for evaluation of acute scrotal pain. Measurements obtained on CDUS included testicular volume of the normal and torsed testicles and the presence or absence of testicular blood flow.¹⁵

All patients with presumed torsion underwent scrotal exploration. During exploration, decisions regarding orchiectomy or orchiopexy were made by the surgeon. All explored patients were managed by detorsion of the torsed testicle with bilateral orchiopexy or orchiectomy.

Outcome measures

The primary outcome measure was the rate of detorsion and testicular viability during surgical exploration. The secondary outcome was the correlation of PVI values in steady-state SCA with torsion surgical findings, considering patients' data from medical and radiology reports, which included the following: testicular volume of both the torsed side and the contralateral side, duration of symptoms, and other torsion clinical findings.

Laboratory analysis

Blood samples were drawn in tubes containing EDTA-K2 (potassium EDTA) anticoagulant from the patients and the control subjects before the surgical intervention. Samples were analyzed within 1 hour.^{16,17} Platelet count (PLT), PVIs (MPV, PDW, and PCT), and total leukocyte count (TLC) were measured using an automated blood cell counter (Sysmex, Japan).

Statistical analysis

Data analysis was performed using IBM SPSS V.24.0. Continuous variables were tested for normality of distribution using the Kolmogorov-Smirnov test. Data were

reported as mean (with standard deviation (SD)) or as median (with interquartile range (IQR)) according to the distribution. Differences in means were compared using Student's t-test for normally distributed data, whereas non-parametric Mann-Whitney U test was used to compare the medians of non-normally distributed data. χ^2 test was used to analyze categorical variables. A receiver operating characteristic (ROC) curve analysis was performed to determine the cut-off values and the area under the curve (AUC) of potential predictive factors. The criterion used in the ROC analysis to define the optimal cut-off value of a predictor was to find the observed value of the predictor that maximized the sum of the predictor's sensitivity and specificity. This is mathematically equivalent to maximizing equally weighted sensitivities and specificities. Multivariable logistic regression analysis was used to identify potential risk predictors. A p value of 0.05 was considered to be the level for a statistically significant difference.

RESULTS

Patient demographic and clinical findings are summarized in table 1. The difference between groups was statistically not significant. Within the groups studied, the median time of symptom duration was 7.5 (IQR 6.2–10.0) and 7.9 (IQR 6.3–13.0) hours for both the patients and the controls, respectively. After a median follow-up of 33.8 (IQR 29.6–37.4) months, 35 (73%) patients with SCA were found to have a viable testis during exploration. The remaining 13 (27%) patients received orchiectomy for non-viable testes. In terms of symptom duration, it was found that a duration of <7 hours (n=34, 71.0%) was statistically significantly correlated with detorsion and testicular viability in patients with SCA ($p<0.001$).

The median PLT, MPV, PDW, and PCT were $236 \times 10^3/\mu\text{L}$ (IQR 197–298), 14.1 fL (IQR 8.4–18.1), 17.9 fL (IQR 15.9–18.4), and 0.34% (IQR 0.18%–0.38%) in torsed patients with SCA, respectively. The median MPV, PDW, and PCT of patients with SCA were significantly higher than those in the control group ($p<0.05$). PLT was higher in the patient group than in the control group but not significantly ($p=0.366$) (table 2). Also, MPV, PDW, and PCT had significant correlation with PLT count ($p=0.247$, $p=0.329$, and $p=0.089$, respectively). Patients with SCA with orchiectomy had higher MPV, higher PCT, and increased PDW count than those in the orchiopey group, which was statistically significant ($p=0.02$, $p=0.03$, and $p<0.001$, respectively).

No statistically significant difference was found between the other clinical findings (scrotal tenderness, swelling, erythema, lower abdominal pain, fever, pyuria, nausea, and vomiting) and PVI values ($p<0.05$).

In the SCA torsed group, the median testicular volume of the torsed side (10.5 (IQR 8.4–12.2) cm^3) was lower than of the contralateral side (14.9 (IQR 12.1–17.4) cm^3), with a significant correlation ($p=0.01$) (table 1). Also, a significant correlation was observed between testicular

Table 1 Patients' demographic and clinical data

Parameter	Patient group (n=48)	Control group (n=46)	P value*
Median age at operation, years†	16.7 (8.5–19.1)	16.2 (10.1–18.8)	0.226
Side of torsion, n (%)			0.257
Right	21 (44)	22 (48)	
Left	27 (56)	24 (52)	
Clinical scrotal findings, n (%)			
Tenderness	44 (92)	45 (98)	0.829
Swelling/erythema	44 (92)	42 (91)	0.659
High-riding testis	39 (81)	38 (87)	0.271
Lower abdominal pain	3 (6)	3 (6.5)	0.121
Nausea and vomiting	3 (6)	4 (9)	0.109
Fever >38.5°C	1 (2)	2 (4)	0.831
Pyuria	1 (2)	2 (4)	0.831
Scrotal Doppler ultrasonography (blood flow), n (%)			0.895
Absent	45 (94)	42 (91)	
Present	3 (6)	4 (9)	
Torsion outcome, n (%)			0.357
Detorsion (fixation)	35 (73)	36 (78)	
Orchiectomy	13 (27)	10 (22)	
MTV of torsed testes, cm^3 †	10.5 (8.4–12.2)	11.3 (9.3–12.3)	0.301
MTV of normal testes, cm^3 †	14.9 (12.1–17.4)	16.1 (12.9–18.5)	0.267

P value: comparison between the patient group and the control group.

*Mann-Whitney U test.

†Values are presented as median (interquartile range, IQR).

MTV, median testicular volume.

volume and detorsion results ($p=0.001$). However, the testicular volume did not have any significant correlation with MPV, PDW, and PCT ($p=0.838$, $p=0.568$, and $p=0.087$, respectively).

The median TLC was significantly higher in the patient group compared with the control group ($p=0.05$) (table 2). At the same time, patients with SCA with orchiopey had a significantly lower TLC than in the orchioectomy group ($p=0.04$).

The ROC curve analysis revealed that the optimal cut-off value for MPV, PDW, PCT, TLC, and symptom duration to predict testicular torsion was 11.5 fL, 12.7 fL, 26%, 10.5×10^3 , and 7 hours, respectively. The corresponding sensitivities were 62.2%, 76.9%, 69.2%, 61.3%, and 84.6%, and the corresponding specificities were 60.0%, 77.1%, 48.6%, 57.1%, and 80.0%. The AUC for MPV, PDW, PCT, TLC, and symptom duration was 0.631 ($p=0.167$), 0.785 ($p=0.003$), 0.590 ($p=0.342$), 0.538 ($p=0.685$), and 0.812 ($p=0.001$), respectively (table 3 and figure 1).

Table 2 Hematological parameters of the studied groups

Parameter	Patient group	Control group	P value*
Platelet count ($\times 10^3/\mu\text{L}$)	236 (197–298)	235 (223–245)	0.366
Mean platelet volume (fL)	14.1 (8.4–18.1)	6.4 (6.3–6.7)	0.03
Platelet distribution width (fL)	17.9 (15.9–18.4)	9.1 (7.9–11.5)	0.000
Plateletcrit (%)	0.34 (0.18–0.38)	0.28 (0.17–0.35)	0.04
Total leukocyte count ($\times 10^3/\text{L}$)	12.6 (9.9–18.8)	10.7 (7.8–12.5)	0.05

P value: comparison between the patient group and the control group.

Values are presented as median (interquartile range, IQR).

*Mann-Whitney U test.

A multivariable logistic regression model for the outcome was constructed and included all of the six predictors (MPV, PDW, PCT, TLC, symptom duration, and testicular volume). Based on this model, symptom duration ($p=0.034$, odd ratio (OR)=1.13, 95% confidence interval (CI) 1.054 to 1.868) and PDW ($p=0.042$, OR=6.586, 95% CI 1.033 to 41.985) were significant predictors of orchiectomy (table 4).

DISCUSSION

The results of the present study showed a significant increase in PDW in testicular torsion patients with SCA, with a significant correlation with detorsion outcome. The PDW values of orchiectomy patients demonstrated a higher significant cut-off of ≥ 12.7 fL for testis survival. Several studies have also reported that PDW is a platelet activation risk marker of developing thromboembolic disorders.¹⁴ Some studies suggest that PDW seems to be a more specific marker of platelet activation than MPV.¹⁴ A key component of this increased PDW is increased platelet production and reactivity, and thus an increased average platelet volume, resulting in an increased PDW. Also, higher concentrations of platelet microparticles have been detected in steady-state SCA.¹⁰ Moreover, increased PDW has been detected in pediatric SCA. PDW also directly measures the changes in platelet size.^{18, 19} Thus, PDW can also be used as a marker of testicular torsion outcomes in patients with SCA.

In the present study significantly higher levels of MPV were observed among the groups.^{8, 11} The MPV values of orchiectomy patients also demonstrated a higher

significant cut-off of 11.5 fL, which is higher than its significant cut-off of 6.5 fL in torsed patients without SCA, as an indicator of testis viability.¹² These findings are mainly related to the increased inflammatory and thrombotic activity, which is associated with increased platelet synthesis in SCA.²⁰ Moreover, torsion in combination with SCA causes marked vascular pathology, alteration in vascular diameter, and induction of coagulation pathway.^{21–23} In addition, hypoxia increases the formation of microthrombi and the production of vasoconstricting endothelin-1.²¹ However, the diagnostic role of MPV in testicular torsion has not been demonstrated in other studies.^{7, 24}

Interestingly, there was no significant correlation between PVI and PLT count.¹¹ However, PVIs are known as indirect indicators of platelet activity and function. Even so, increased platelet activity with higher PVIs is more likely responsible for the endovascular complications in SCA.^{23–25} The lack of this significant correlation may be related to the accelerated production of megakaryocytes, resulting in an increase in platelet number. These larger platelets are metabolically more active owing to the presence of thromboxane A2 and soluble P-selectin.^{26, 27} However, a higher PLT was found in these torsed patients with SCA, which may have resulted directly from increased platelet synthesis. We also reported increased PCT with its implications on exploration outcomes in such patients. This is not consistent with Mutlu *et al's*²⁸ observations, who found PCT values are low with ignorable effect on thrombosis development. It is noteworthy that some studies failed to confirm MPV,

Table 3 Prediction of testicular torsion outcome according to the cut-off values of MPV, PDW, PCT, TLC, and symptom duration

Parameter	Cut-off value	AUC	95% CI	P value	Sensitivity (%)	Specificity (%)
MPV	11.5 fL	0.631	0.451 to 0.811	0.167	62.2	60.0
PDW	12.7fL	0.785	0.615 to 0.955	0.003	76.9	77.1
PCT	26%	0.590	0.416 to 0.765	0.342	69.2	48.6
TLC	10.5×10^3	0.538	0.362 to 0.715	0.685	61.3	57.1
Symptom duration	7 h	0.812	0.658 to 0.966	0.001	84.6	80.0

AUC, area under curve; CI, confidence interval; MPV, mean platelet volume; PCT, plateletcrit; PDW, platelet distribution width; TLC, total leukocyte count.

Figure 1 Scatterplot of the observed values of the platelet distribution width (PDW) and symptom duration. Testicular torsion outcome in patients with steady-state sickle cell anemia is indicated by color (black: orchietomy; violet: orchiopexy). The line plotted in figure 1 was calculated by adding the fit line at total (R^2 linear=0.217). PDW, platelet distribution width.

PDW, and PCT as predictors of testicular torsion, probably due to differences in the laboratory methods and/or equipment used for analysis.^{16 29}

Along with providing MPV, PDW, and PCT, complete blood count includes other biomarkers that are often investigated, such as TLC. In this study, the predictive value of TLC was demonstrated in accordance with Bitkin *et al*,⁸ where TLC was significantly increased in patients with torsion as a result of cremasteric muscle tissue torsion, which is associated with severe local hypoxia and reduced microcirculation. The prognostic value of leukocyte subtypes and the ratio of these biomarkers (neutrophils, lymphocytes, and monocytes) concerning inflammatory response have been confirmed in other studies.⁸

The association between testicular torsion and testicular atrophy is well documented in this report. Moreover, testicular volume seems to be affected by higher PVI values, but without a significant correlation. This correlation indicates that the higher production

of PVI by the activated platelet, induced by testicular torsion, is associated with high platelet endothelin-1 production, which in turn may result in testicular ischemia. This relationship was demonstrated experimentally by the reduction of ischemia-related testicular damage by administration of antiplatelet activating factor.³⁰ There are also other factors induced by SCA pathogenic mechanisms that could invalidate such correlation (eg, increased intratesticular vascular microthrombus and activation of the intrinsic coagulation pathway).^{9 10 30} Testicular atrophy in SCA with torsion is characterized by progressive necrotic testicular parenchyma and loss of testicular volume.⁴

In the present study, patients with symptom duration over 7 hours before detorsion had a significant correlation for predicting viability of the testicle preoperatively. This result is consistent with those of previous studies that reported testicular atrophy was significantly more common with durations longer than 6 hours.⁴ Nevertheless, symptom duration had no significant impact on the PVI in this torsion group. This may be because not all patients with a longer duration had complete testicular volume loss, which may be explained by intermittent torsion during the time between onset of symptoms and detorsion, or due to spotty scattered testicular parenchyma injury secondary to both interstitial edema and compression of the microcirculatory system.^{31 32}

Despite the relatively low numbers of testicular torsion in the study, these values should be considered an acceptable result for statistical correlation with hematological markers. Moreover, this report confirmed an independent poor outcome of torsion exploration in patients with SCA with both increased PDW and longer symptom duration. Interestingly, PDW has the advantage that it can be routinely measured using all hematological analyzers. Additionally, it can be easily included in routine use with various demographic clinical findings as a predictive factor for assessment of acute scrotal pain in SCA.

There are several limitations within this report. One is the study's relatively small size sample. Another potential limitation is that we did not include an assessment of other causes of acute scrotum diseases, such as epididymitis or appendicular torsion, which are commonly misdiagnosed with testicular torsion. The diagnosis in

Table 4 Multivariable analysis of the predictive factors for testicular torsion outcome

Predictors	OR	95% CI		P value
		Lower	Upper	
Mean platelet volume (fL)	0.232	0.027	1.980	0.182
Platelet distribution width (fL)	6.586	1.033	41.985	0.042
Plateletcrit (%)	0.433	0.073	2.578	0.358
Total leukocyte count ($\times 10^3/L$)	1.343	0.123	14.712	0.809
Symptom duration (h)	1.13	1.054	1.868	0.034
Testicular volume (cm^3)	2.574	0.245	27.015	0.431

CI, confidence interval; OR, odd ratio.

the present study was established mainly after surgical exploration.

In conclusion, this study shows that an increased PDW in SCA with testicular torsion is a poor platelet prognostic marker, which is associated with testicular salvage rate. Furthermore, longer symptom duration with increased PDW value may be diagnostic parameters for high-risk testicular atrophy in SCA with acute scrotum. Therefore, the use of PDW may help physicians in the diagnosis of a high-risk group of patients with SCA with acute scrotum, which is a candidate for emergency scrotal exploration. However, there is a great need for further multicenter investigations or prospective clinical research with a larger sample size to confirm this relationship.

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Data availability statement Data are available upon reasonable request. All data relevant to the study are included in the article or uploaded as supplementary information.

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ORCID iD

Mazen Ahmed Ghanem <http://orcid.org/0000-0002-2412-7212>

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