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Original Research Article

Comparative Analysis of Platelet Count Estimation by Peripheral Smear Method and Automated Hematology Analyser Method in Thrombocytopenic Patients

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Abstract

Aim: To estimate and correlate the platelet count by automated cell counter and manual method.

Materials and Methods: The study was Prospective Cross- Sectional Study, carried out in the Department of Pathology, Adesh Medical College and Hospital Shahabad, Kurukshetra, on a group of 200 patients (125 males and 75 females) from 1st March to 31st May, 2024 in 3 months duration. All the obtained data were handled with confidentiality. Blood samples were collected from all age group by venipuncture in ethylene diamine tetra-acetic acid (EDTA) tubes following complete aseptic precautions. Smears will be prepared immediately and stained using Leishman's stain following standard protocol. Platelets were counted in ten oil immersion fields and the count was multiplied by 15000 to obtain platelet count in lacs/mm^3. The platelet count was also be determined by automated hematology analyzer.

Results: We observed 63% of the patients were male and rest 37% were female with male: female of 1.7:1. Most of the patients belong to the age group of 30- 40 years i.e. 21%. The mean platelet count on automated analyzer was 67.5 ± 36.4 x10^3 /µL whereas the mean platelet count verified on peripheral smear was 106.1 ± 61.5 x 10^3/µL with a significant difference between the two groups (p-value <0.001). On manual examination, we observed that 23% of the patients which were previously diagnosed as thrombocytopenic on automation were found to be adequate in manual method i.e. pseudo-thrombocytopenia cases. We also evidenced that when examined on peripheral smear the actual platelet count was actually higher than automated count for most of the thrombocytopenic cases.

Conclusion: In thrombocytopenic cases, verifying the platelet count generated by automated hematology analyzers through peripheral smear examination is imperative, particularly when abnormal platelet morphologies, such as giant platelets and platelet clumps are present. This confirmation is critical prior to initiating treatment, as it helps avoid unnecessary diagnostic procedures and inappropriate therapeutic interventions.

Keywords: Platelets, Thrombocytopenia, Hematology Analyzer.

Introduction

Platelets are formed elements of blood [1]. Platelets are small colorless, non- nucleated, discoid shape. Platelets play a key role in homeostasis and thrombosis [3]. Platelets are adapted to adhere to damaged blood vessels, aggregate on one another and facilitate generation of thrombin [4]. Normal platelet count in a healthy person is 1.5- 4.0 lakh/mm^3 of blood [5]. The accurate platelet count estimation has an important role in diagnosis and treatment of thrombocytopenia cases. The reliability of platelet count is highly desired where the platelet transfusion is necessary [6]. Thrombocytopenia is commonly associated with various conditions like bacterial sepsis, renal failure, leukemia, malignancy, after chemotherapy etc [7].

Estimation of platelet count is frequently recommended especially during dengue fever season [8]. Platelet count is important diagnostic

tool so it is necessary to count the platelets accurately [3]. Platelets are counted by two approaches i.e. manual method and automated method. Manual method by using diluting fluid like 1% ammonium oxalate in Neubauer chamber and also slide method with Leishman stain, these methods are simple, economical and suitable if done in proper manner [9].

However, with the advent of automation, hematology analyzers have taken over in day to day practice from semi- automated to completely automated machines, based on the principles of impedance, flow cytometry and optical fluorescence [3]. Manual methods are time consuming, subjective and tedious. [10]. Automated hematology analysers though rapid in giving results, at times give erroneous values in the presence of giant platelets, platelet clumps, fragmented and microcytic red blood cells [11].

ISLH (International society for laboratory in hematology) and ICSH (International council for standardization in hematology) recommend the estimation of platelet count as a reference modality for calibration of automated analyzer. For this a flow cytometry and experienced technicians are required [5,12].

Occasionally platelet satellitism may give wrong results by automated cell counter in ethylene diamine tetra acetic acid (EDTA) samples. Results of automated counters can't be totally relied in severe thrombocytopenia [13].

Aims and Objectives

1. To estimate the platelet count by automated cell counter and manual method.
2. To correlate the platelet counts done by automated cell counter and manual method.

Material and Method

The study was Prospective Cross- Sectional Study, carried out in the Department of Pathology, Adesh Medical College and Hospital Shahabad, Kurukshetra, on a group of 200 patients (125 males and 75 females) from 1st March to 31st May, 2024 in 3 months duration. All the obtained data were handled with confidentiality. Blood samples were collected from all age group by venipuncture in ethylene diamine tetra-acetic acid (EDTA) tubes following complete aseptic precautions. Smears were prepared immediately and stained using Leishman's stain following standard protocol.

Platelets were counted in ten oil immersion fields and the count was multiplied by 15000 to obtain platelet count in lacs/mm^3. The platelet count was also determined by automated hematology analyzer.

Qualitative variables were described as frequency, and quantitative variables were measured as mean and standard deviation and keeping the 95% confidence interval and p-value of <0.05. T- Test was applied to compare the mean platelet count obtained by both methods.

Inclusion Criteria – All blood samples sent for assessment of platelet count and found to have low platelet count by peripheral smear method during the study period.

Exclusion Criteria – Hemolyzed and clotted samples were excluded from study.

Results: We studied 200 patients with thrombocytopenia i.e. <150 x $10^3/\mu L$ platelet count on automated cell counter. We observed 63% of the patients were male and rest 37% were female with male: female of 1.7:1 (Figure 1). Most of the patients belong to the age group of 30- 40 years i.e. 21% (Figure 2). The mean platelet count on automated analyzer was 67.5 ± 36.4 x10^3 /µL whereas the mean platelet count verified on peripheral smear was 106.1 ± 61.5 x $10^3/\mu L$ with a significant difference between the two groups (p-value <0.001) (Table 1, Figure 3).

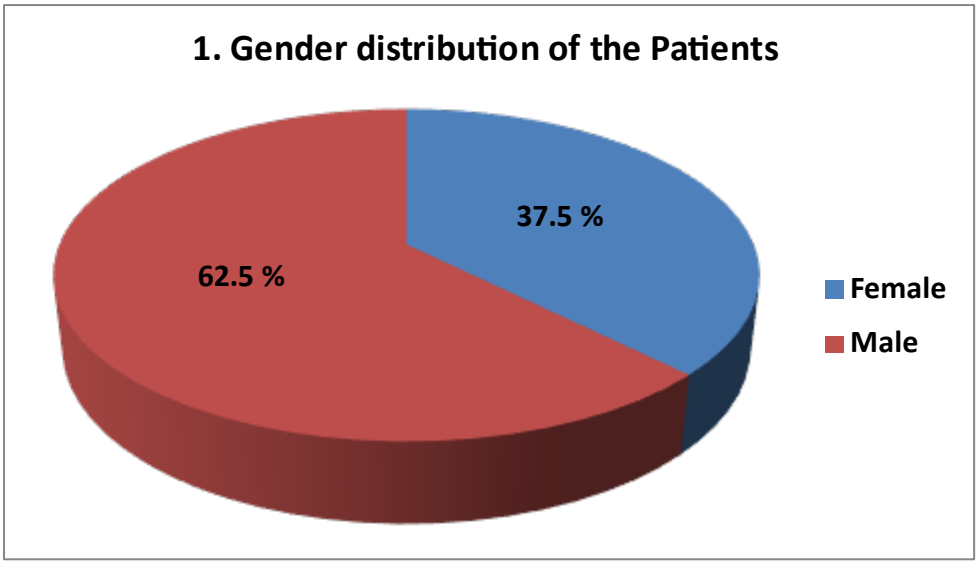


Figure 1: Gender wise distribution of the patients

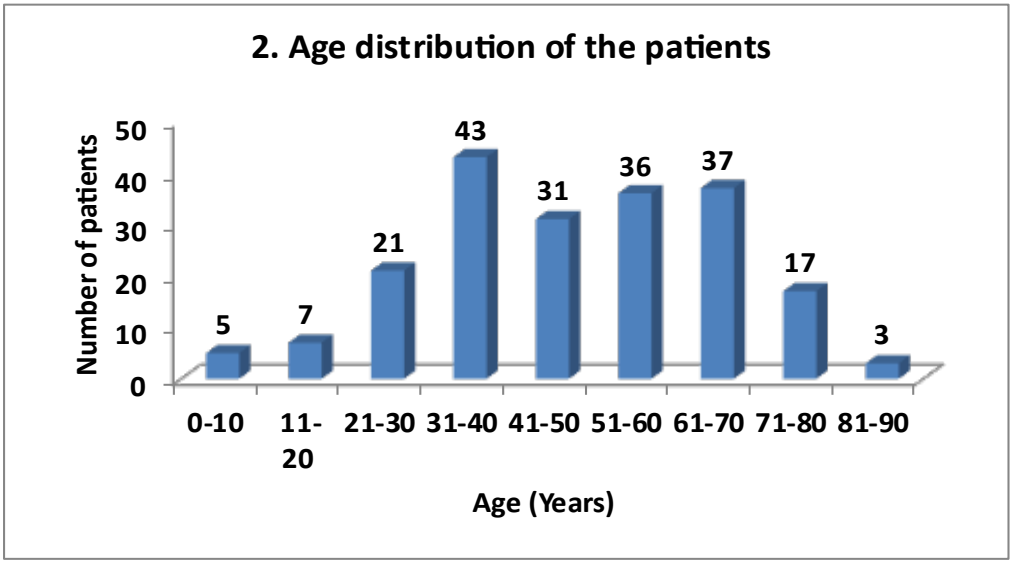


Figure 2: Age wise distribution of the patients

Table 1: Comparison of mean platelet estimation by manual peripheral blood smear examination and automated cell counter

Parameter	N	Mean (x1000/µl)	Std. Deviation	t- Value	P Value
Automated Method	200	67.5	36.4	7.63	<0.001*
Manual Method	200	106.1	61.5		

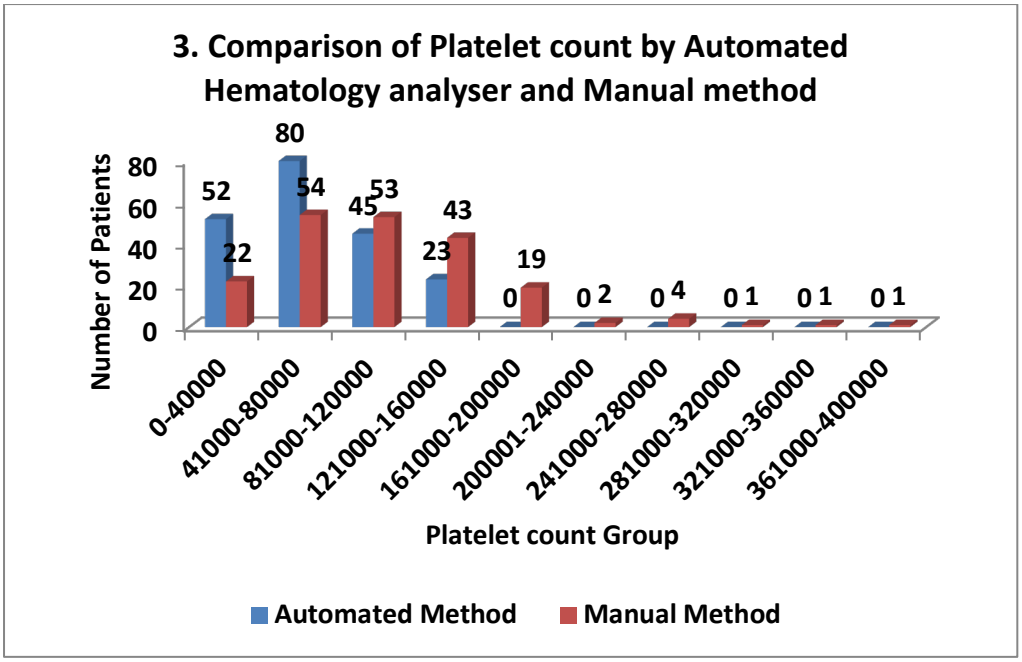


Figure 3: Comparison of platelet counts on automated hematology analyser and manual method.

On manual examination, we observed that 23% of the patients which were previously diagnosed as thrombocytopenic on automation were found to be adequate in manual method i.e. pseudo-thrombocytopenia cases (Figure 4). We also evidenced that when examined on peripheral smear the actual platelet count was actually higher than automated count for most of the thrombocytopenic cases.

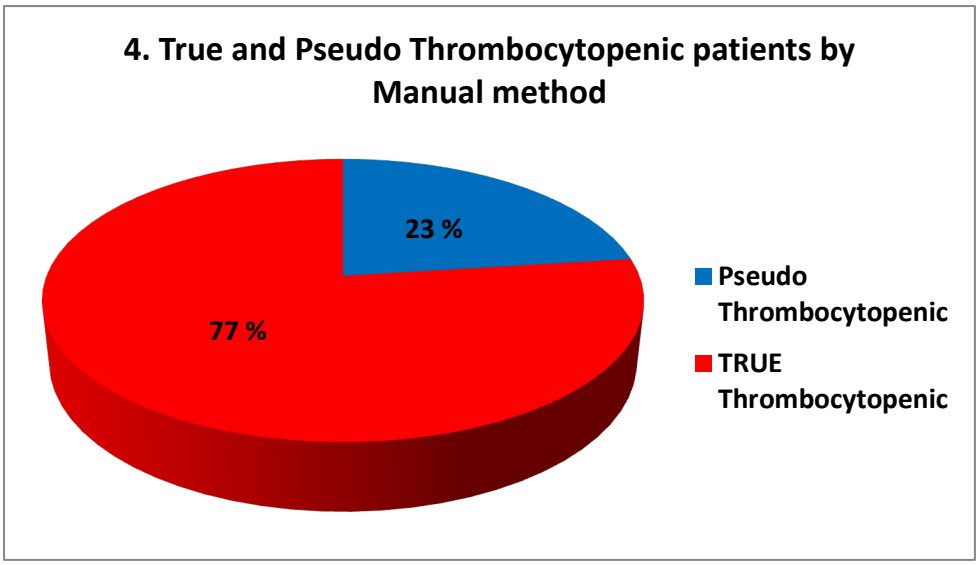


Figure 4: True and pseudo- thrombocytopenic patients by manual method

The causes of pseudo thrombocytopenia when confirmed on peripheral examination were the following:26.2 % of the patients had platelet clumps,38.1% of the patients had giant platelets, and 35.7 % of patients had both giant platelets and platelet clumps (Figure 5)

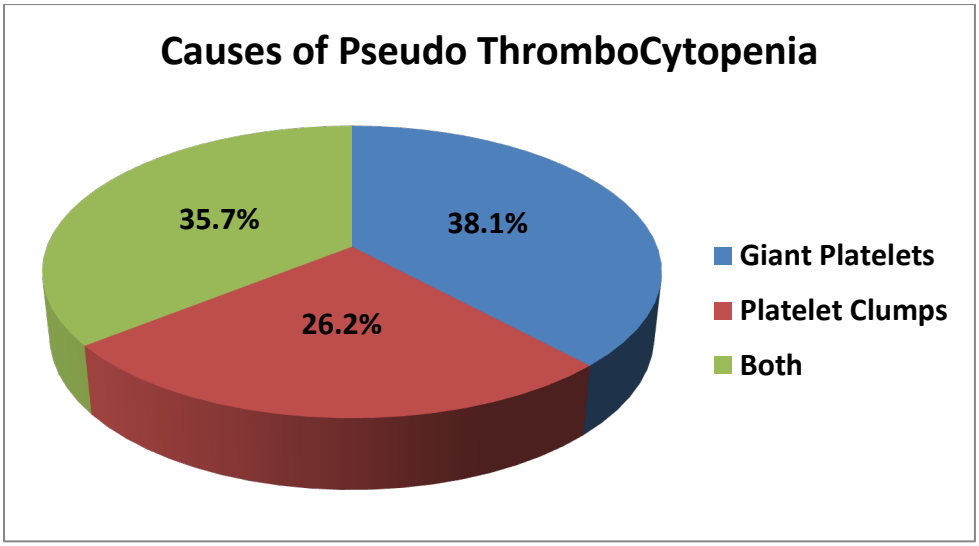
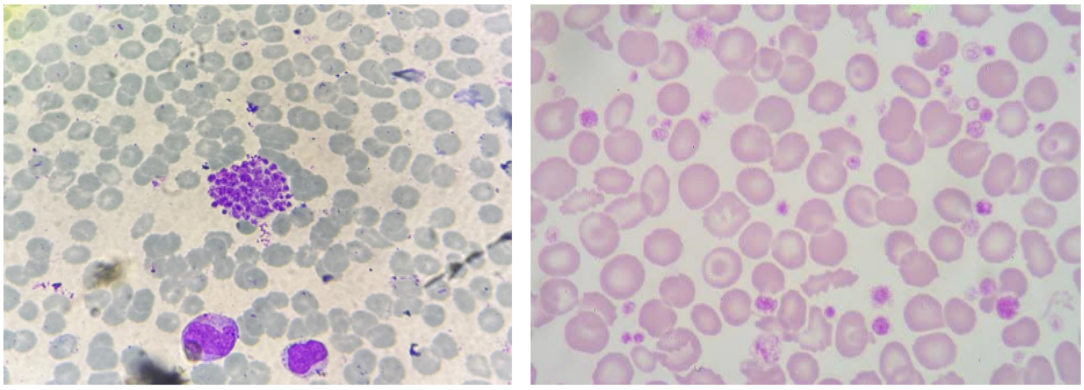


Figure 5: Percentages of causes of pseudo thrombocytopenia



A) B)

Figure 6: Peripheral blood smear (PBS) under 100x oil immersion showing A) platelet clumps B) giant platelets

We have divided the thrombocytopenic cases according to severity i.e. mild, moderate and severe thrombocytopenia. On automation, out of 200 thrombocytopenic cases 34(17%) mild, 94 (47%) moderate and 72(36%) severe thrombocytopenic cases were there whereas by manual method only 154 cases out of 200 were thrombocytopenic with 40 (26%) mild, 82 (53.2%) moderate and 32 (20.8%) severe thrombocytopenic cases (Figure 7).

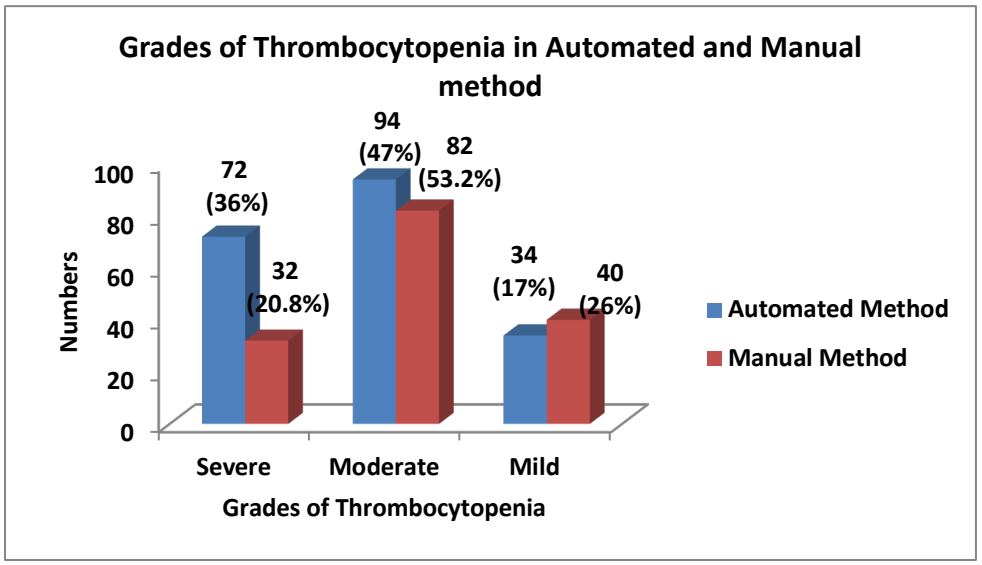


Figure 7: Comparison of grades of thrombocytopenia in automated and manual method

Discussion

In the past few years, automated hematology analyzers which are used for both analytical and whole blood cells description have seen substantial advancements. Because of this, manual procedure in hematology labs for regular testing has been increasingly losing their importance [14]. Despite the fact that hematology analyzers frequently yield accurate platelet counts, their accuracy has been in doubt when there is low platelet count, platelet abnormalities, or infiltration from fragments that resemble platelets [15]. When an automated platelet count is low or flagged, the calculation of platelet counts from the manual method counting by examining blood smears should be the gold standard, since no machine, no matter how costly or effective, can completely replace human judgement [16].

We observed that out of 200 patients 63% were male and rest 37% were female and male to female ratio was 1.7:1. Ajeet et al., [17] studied 100 patients included 63% were and 37% were female with an approximate male-female ratio 1.7:1 Gogoi et al., [18], observed 797 thrombocytopenic patients with 71% male and 29% female with (male: female =2.44:1). Another study Tariq et al., [19] studied 60 patients including 31 female and 29 males with an approximate male-female ratio 1:1. Castromayor et al., [16] also observed 384 adult patients with thrombocytopenia, with an approximately 1:1 ratio based on sex.

In the present study, the majority of patients (21.5%) were aged 30–40 years, followed by 18.5% in the 60–70-year range. Similarly, Ajeet et al. [17] found that 34% of their cases were in the 30–40-year group, with the 20–30 year group being the next most common. Gogoi et al. [18] reported comparable findings, with 22.5% of cases in the 30–40-year group, followed by 19% in the 20–30 year group. In contrast, Castromayor et al.[16] observed that thrombocytopenia was most prevalent in individuals aged 60–80 years, affecting 36% of patients. Tariq et al. [19] noted a mean patient age of 43.7 years.

The mean platelet count measured by automated analyzers was $67.5 \pm 36.4 \times 10^3/\mu L$, while the count on peripheral smear was $106.1 \pm 61.5 \times 10^3/\mu L$, showing a significant difference between the two methods ($p < 0.001$). This variation is mainly due to the presence of platelet clumps and giant platelets in the samples. Ajeet et al. [17] reported that the mean platelet count on automated analyzers was $85.46 \pm 38.81 \times 10^3/\mu L$, compared to $92.13 \pm 38.30 \times 10^3/\mu L$ on peripheral smear, also demonstrating a significant difference ($p < 0.0001$). Their study focused only on the presence of giant platelets in the samples. Castromayor et al. [16] found that the mean platelet count using automated methods was around $76 \pm 45 \times 10^9/L$, while manual counting yielded $170 \pm 109 \times 10^9/L$, with a significant difference ($p < 0.005$). Tariq et al. [19] observed a mean platelet count of $58 \pm 28 \times 10^9/L$ on automated analyzers, compared to $117 \pm 13 \times 10^9/L$ on peripheral smear, with a significant difference ($p < 0.001$). Their study accounted for samples with both platelet clumps and giant platelets, which contributed to the observed differences. We noted 23% pseudo-thrombocytopenia cases. Ajeet et al., [17] documented 10% pseudo-thrombocytopenia cases inclusion of only giant platelet cases. While Tariq et al., [19] showed 42% pseudo-thrombocytopenia patients owing to the inclusion of the cases with platelets clumps apart from giant platelets cases. However, Gogoi et al., [18] documented 9.8 % pseudo-thrombocytopenia cases in their study. 38.2 % of the cases showed giant platelets and 26.2% cases showed platelet clumps. Whereas Ajeet et al., [17] Tariq et al., [19] Gogoi et al., [18] reported giant platelets in 29%, 39% & 11.5% of the cases respectively. However, Tariq et al., [19] also reported platelet clumps in their study. Ajeet et al. [17] evaluated 100 cases of thrombocytopenia, reporting 38% as mild, 41% as moderate, and 21% as severe based on automated counts. However, when assessed through peripheral smear, only 90 cases were identified, with 43.3% classified as mild, 41.1% as moderate, and 15.6% as severe. In comparison, the current study found 17% mild, 47% moderate, and 36% severe cases on automation, while peripheral smear results showed 26% mild, 53.2% moderate, and 20.8% severe cases. These findings highlight a shift in thrombocytopenia grading, as peripheral smear detected some cases with normal platelet counts that were previously classified as thrombocytopenic by automation.

Conclusion

Thrombocytopenia occurs with similar frequency in both sexes, predominantly affecting middle-aged individuals. In thrombocytopenic cases, verifying the platelet count generated by automated hematology analyzers through peripheral smear examination is imperative, particularly when abnormal platelet morphologies, such as giant platelets and platelet clumps are present. This confirmation is critical prior to initiating treatment, as it helps avoid unnecessary diagnostic procedures and inappropriate therapeutic interventions. Therefore, peripheral smear evaluation remains the gold standard for precise platelet count assessment.

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