Role of erythrocytes and platelets in the hypercoagulable status in polycythemia vera through phosphatidylserine exposure and microparticle generation

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Summary
The development of thrombosis in polycythemia vera (PV) involves multifactorial processes including pathological activation of blood cells. Release of microparticles (MPs) by activated cells in diseases is associated with thrombotic risk, but relatively few data are available in PV. The aim of the present study was to investigate the increase in MP release and exposure of phosphatidylserine (PS) on the outer membrane of MP-origin cells in patients with PV, and to analyse their procoagulant activity (PCA). PS-positive MPs and cells were detected by flow cytometry, while PCA was assessed with clotting time and purified coagulation complex assays. We found that PV patients had increased circulating lactadherin+ MPs, which mostly originating from erythrocytes, platelets, granulocytes, and endothelial cells, as well as increased PS exposing erythrocytes/platelets as compared to secondary polycythemia patients or healthy controls. These PS-bearing MPs and cells were highly procoagulant. Moreover, lactadherin competed factor V and VIII to PS and inhibited about 90% of the detected PCA in a dose-response manner while anti-TF antibody did no significant inhibition. Treatment with hydroxyurea is associated with a decrease in PS exposure and lactadherin+ MP release of erythrocytes/platelets. Our data demonstrate that PV patients are characterised by increased circulating procoagulant MPs and PS exposing erythrocytes/platelets, which could contribute to the hypercoagulable state in these patients.

Keywords
Phosphatidylserine, microparticles, polycythemia vera, lactadherin

Introduction
Polycythemia vera (PV) is a chronic myeloproliferative disease in which arterial and venous thrombosis represent the main cause of morbidity and mortality (1). The mechanisms underlying the thrombosis in PV are largely elusive and likely to be multifactorial. Increased haematocrit and whole-blood viscosity, quantitative/qualitative abnormalities of blood cells, interactions between blood cells, and impaired fibrinolytic activity may all contribute to the thrombotic status in PV (2-4). However, causal relationships between any of these specific abnormalities and thrombosis have not been clearly established in that the clinical and pathogenetic significance of previously reported data has often been conflicting or difficult to interpret. Some previous studies showed that haematocrit level was strikingly correlated to the occurrence of thrombosis in PV (2). Nevertheless, this paradigm has been challenged recently by a retrospective analysis that fails to show any correlation between the haematocrit level and the occurrence of major cardio-vascular events (5). In addition, there is no known correlation between a particular platelet defect and either a bleeding or thrombotic tendency in patients with PV though biochemical and functional abnormalities of platelets have been described in PV (6). Thus, exploring and understanding the additional mechanisms involved in thrombotic status in PV are necessary and may be helpful in the development of new therapies in PV.

Phosphatidylserine (PS), a negatively charged phospholipid, is an important cofactor in a number of reactions in the coagulation cascade, including the activation of factor (F)X to FXa and the generation of thrombin from prothrombin. PS is normally localised in membrane leaflets that face the cytosol and is inaccessible to blood coagulation proteins (7, 8). However, when apoptosis occurs or cells are activated, membrane phospholipid asymmetry is lost and PS is externalised to the outer membrane (9). In addition, loss of membrane lipid asymmetry is often accompanied by blebbing and subsequent shedding of microparticles (MPs) from the cell surface (10). The blood MPs are vesicles with diameter between 0.1-1 μm and ab-
normally display PS on their outer leaflets. MPs in blood can originate from platelets, erythrocytes, leukocytes, monocytes and endothelial cells (11). PS exposure and MP release by activated cells have been reported in patients associated with thrombotic risk, such as sickle cell disease, diabetes, essential thrombocythemia, and nephrotic syndrome (12-15). Recent reports showed that PV patients had higher percentage of PS exposure on erythrocytes and platelets than healthy subjects (16, 17). However, the studies did not evaluate the release of MPs and the associated PCA of these cells. In view of these facts, we speculate that high levels of MPs of erythrocyte and platelet origin could also be detectable in blood of PV patients and may therefore contribute to the prothrombotic status.

Lactadherin, a glycoprotein of the milk-fat globule membrane, contains tandem C domains with homology to the PS-binding domains of blood-clotting factors V and VIII. It binds to phospho-L-serine in a stereoselective manner, independent of Ca\(^{2+}\) concentration and membrane phosphatidylethanolamine content (18). Our previous study demonstrated that lactadherin is a more precise probe for PS on cell and MP surface compared with annexin V (9, 19). Moreover, our previous work also proved that lactadherin is an efficient anticoagulant through competing with factors V and VIII for membrane-binding sites (20, 21). Thus, we used lactadherin to identify PS exposure on MPs and cells and their association with PCA in the study.

The present study focused on MP release and PS exposure by erythrocytes/platelets and aimed to investigate the role of blood MPs and PS exposure cells in the pathogenesis of thrombosis in PV.

**Patients, materials, and methods**

**Patients**

Twenty-three consecutive PV patients (12 males and 11 females, median age: 56, range: 41-74 years) and 21 secondary polycythemia (SP) patients (10 males and 11 females, median age: 59, range: 42-72 years) enrolled at the Department of Haematology in the First Affiliated Hospital of Harbin Medical University entered the study. All PV patients had the JAK2\(^{V617F}\) mutation and satisfied the 2008 World Health Organisation criteria for PV (22). Patients who did not meet the criteria for PV and with obvious secondary causes, such as smoking or chronic lung disease, were diagnosed as secondary polycythemia. In addition, 10 healthy volunteer subjects were investigated in our study. None of the healthy controls were taking any drugs affecting the coagulation system at the time of blood collection. There were no significant differences in age or sex distribution among the polycythemia vera, secondary polycythemia, and healthy subject groups. Ethics approval for the study had been obtained and each person provided informed consent. Among the PV patients, five were receiving treatment with hydroxyurea (HU), three with phlebotomy, five with aspirin and the remaining 10 PV patients were not receiving any treatment. Blood samples were obtained when patients were admitted. In addition, 10 untreated and two phlebotomy-treated PV patients, who adopted HU therapy alone later, provided a HU-treated blood sample during a subsequent visit to the outpatient clinic. HU was administered according to haematologic protocols currently used in our hospital (The starting dose is 15-20 mg/kg/day until response is obtained. Then a maintenance dose is administered to keep the response without reducing leukocyte counts below 2500\(\times10^3/\mu l\)). The median duration of HU therapy was 8.6 months (range 1.5-12 months). No diabetes, malignant tumour, iron deficiency, active or chronic infection was presented in these patients. Characteristics of all the study participants are summarised in Table 1.

**Materials**

Truecount Tube (Cat. No. 340334), cell-specific monoclonal antibodies (MoAbs) against CD41a for platelets (clone HIP8), CD31 for endothelial cells (clone L133.1), CD235a for red blood cells (clone GA-R2), CD15 for granulocytes (clone HI98), CD142 for endothelial cells (clone HFT-1) and mouse IgG1/IgG2a (clone

| Table 1: Characteristics of study subjects included in the study. |  |
|---|---|---|
| Healthy subjects | Secondary polycythemia | Polycythemia vera |
| Male/female | 5/5 | 10/11 | 12/11 |
| Age (years) | 54.5 ± 8.54 | 57.85 ± 10.28 | 56.73 ± 8.79 |
| Erythrocytes (\(\times10^9/\mu l\)) | 4.46 ± 0.48 | 5.90 ± 0.58* | 7.21 ± 1.12* |
| Platelets (\(\times10^9/\mu l\)) | 220.8 ± 54.36 | 204.87 ± 80.43 | 418.64 ± 205.73*# |
| White blood cells (\(\times10^9/\mu l\)) | 6.49 ± 1.52 | 8.55 ± 4.03 | 13.28 ± 6.94*# |
| Neutrophils (\(\times10^7/\mu l\)) | 4.08 ± 0.93 | 5.89 ± 3.90 | 9.03 ± 4.72# |
| Hemoglobin (g/l) | 139.60 ± 13.17 | 188.21 ± 16.24* | 198.66 ± 25.21* |
| Hematocrit (%) | 41.55 ± 2.93 | 55.37 ± 4.81* | 58.86 ± 6.95* |
| Patients’ therapy | - | - | - |
| Hydroxyurea | - | - | 13 (57%) |
| Phlebotomy | - | - | 5 (22%) |
| Aspirin | - | - | 3 (13%) |
| History of thrombosis | - | - | 5 (22%) |

Data are presented as mean ± SD or number (%). *p<0.05 vs. healthy subjects. #p<0.01 vs. secondary polycythemia patients.
Protein purification and labelling

Lactadherin was purified from bovine milk and labelled with Alexa Fluor 647 or Alexa Fluor 488 as described in previous research (23, 24). The ratio of fluorescein to lactadherin was 1.2/1 or 1.1/1.

Blood collection and preparation of erythrocytes/platelets/MPs

Blood samples were obtained from venous blood using a 21-gauge needle into 3.2% sodium citrate (9 volume of blood to 1 volume of anticoagulant) after discarding the first 3 ml. Platelet-rich plasma (PRP), erythrocytes and platelet-free plasma (PFP) were prepared immediately after blood collection. PRP and erythrocytes were prepared by centrifugation for 13 minutes (min) at 200 x g at room temperature, without storage. PFP was prepared by a two-step centrifugation procedure: initially at 1,500 x g for 20 min at room temperature and then 13,000 x g for 2 min at room temperature. Then PFP aliquots of 0.25 ml were used immediately or snap-frozen in liquid nitrogen and stored at -80°C. MPs were obtained by ultracentrifugation from PFP as previously described (25, 26).

Briefly, 250 µl of frozen PFP were thawed on ice for 1 hour and then centrifuged for 90 min at 20,000 x g at 4°C, subsequently, 225 µl of supernatant were removed. The remaining 25 µl MP pellet was washed twice (20,000 g for 90 min at 4°C) and resuspended in 125 µl Tyrode's buffer (137 mM NaCl, 2.7 mM KCl, 11.9 mM NaHCO3, 0.42 mM Na2HPO4, 1 mM MgCl2, 2 mM CaCl2, 5.5 mM glucose, 5 mM Hapes, 0.35% BSA, pH 7.4).

Flow cytometric analysis of PS exposure on erythrocytes/platelets

Erythrocytes/platelets were adjusted to 0.5-1 x 10^9/ml in Tyrode's buffer, and incubated with 5 µl of Alexa Fluor 488-lactadherin for 10 min at room temperature, shielded from light. Then samples were analysed using flow cytometry. Ten thousand events per sample were acquired and analysed with BD FACSDivA Software.

Procoagulant activity (PCA) and inhibition assays of MPs/erythrocytes/platelets

PCA was determined by a one-stage recalcification time assay as described previously (21). Erythrocytes/platelets were adjusted to 1 x 10^9/ml and 1 x 10^8/ml with Tyrode's buffer, respectively. MP suspension was prepared as mentioned above. All reagents were preheated at 37°C prior to the experiments. A volume of 100 µl of cell or MP suspension was incubated with equal volume of MP-free human plasma (obtained by ultracentrifugation of whole blood, anticoagulated with 3.2% sodium citrate, 1:9, v/v) for 180 seconds (sec) at 37°C. After 100 µl warmed 25 mM CaCl2 was added, coagulation time of the mixture was recorded by a coagulometer (Amelung KC4A, Labcon, Heppenheim, Germany). Each sample was tested in duplicate. For the inhibition assays, a total of 100 µl of cell or MP suspension was incubated with 50 µl lactadherin (final concentration 128 nM) or anti-tissue factor (final concentration 40 µg/ml) for 10 min at 37°C. Then 100 µl of MP-free plasma were added in the mixture. After incubation for 180 sec at 37°C, 50 µl of 50 mM CaCl2 was added. Then clotting time of the mixture was recorded.

Intrinsic, extrinsic FXase and prothrombinase formation and inhibition assays

The formation of intrinsic, extrinsic FXa and prothrombinase in the presence of RBCs/platelets/MPs was performed as previously described (13, 28). For the intrinsic FXase formation assays, 1 x 10^5 erythrocytes or 1 x 10^4 platelets or 10 µl of MP suspension

X40/X39) were from Becton Dickinson Biosciences (San Jose, CA, USA). All monoclonal antibodies were labelled in our laboratory with Alexa Fluor 647 or Alexa Fluor 488. Lactadherin and Alexa Fluor 647/Alexa Fluor 488 labelled lactadherin were prepared in our laboratory. Standard fluorescent beads (0.5, 0.9, 3.0 µm) were used for size calibration and to set the gate of MP detection at a diameter from 0.5 to 1 µm, as previously described (27). All dilution buffers were filtered using 0.22 µm filters (Millipore, Billerica, MA, USA). To differentiate MPs from events due to noise, MPs were identified as PS positive particles, specifically labelled with lactadherin. Origin of MPs was defined by co-labelling with lactadherin and cell-specific antibodies. Count beads were discriminated from the MP population on light scatter and counted using a separate gate. Acquisition was stopped after 10,000 events were counted in the MP gate. The number of MPs per microliter of plasma (n) was calculated using the following formula: n = (C x Beads_diluted)/(Beads_counted x sample volume) x 24, where C represents the number of positive events with the background signal subtracted and 24 is a dilution factor.
(prepared as described above) were incubated with 1 nM FIXa, 130 nM FX, 0.2 nM thrombin, and 5 nM FVIII in FXa buffer (1 ml 10x TBS, 200 µl 10% BSA, 8.8 ml ddH₂O) at 25°C for 5 min followed by addition of EDTA (7 mM, final concentration) to stop the reaction. Immediately after the addition of 10 µl chromogenic substrate S-2765 (0.8 mM), production of FXa was determined by measurement of absorbance at 405 nm on a Universal Microplate Spectrophotometer (PowerWave XS, Bio-Tek, Winooski, VT, USA) in kinetic mode. The measurement lasted 15 min, and the read interval was 11 sec. Each sample was tested in triplicate. Results were measured against the rate of substrate cleavage of a standard dilution of FXa. The means of generated FXa for all time points were analysed. For the extrinsic FXa formation assay, cells or MPs were mixed with 130 nM FX, 1 nM FVIIa and 5 mM Ca²⁺. After 5 min incubation at 25°C, the reaction was stopped with EDTA and the quantity of FXa formed was determined as described above. In the prothrombinase formation assay, cells or MPs were incubated with 1 nM FVa, 0.05 nM FXa in the presence of 1 μM prothrombin and 5 mM Ca²⁺ in prothrombinase buffer (1 ml 10x TBS, 50 µl 10% BSA, 8.95 ml ddH₂O) at 25°C for 5 min. After addition of EDTA and chromogenic substrate S-2238 (0.8 mM), thrombin production was assessed as described in the FXase formation assay. Results were evaluated against the rate of substrate cleavage from a dilution curve of thrombin. For the inhibition assays of intrinsic FXase and prothrombinase production, cells and MPs were incubated with varying concentrations of lactadherin (0-128 nM) at 25°C for 10 min before reaction. The mixture was then incubated with the specified clotting factors according to the above protocols. The amount of FXa or thrombin was measured as described above.

**Statistical analysis**

Data are presented as mean ± standard deviation (SD), statistical differences between groups were determined with the non-parametric Kruskal-Wallis or Mann-Whitney U tests as appropriate because the data had a non-Gaussian distribution. Bivariate correlations were performed using the Spearman's rank correlation. Paired sample analysis was done using the Wilcoxon’s rank test. P-values of 0.05 or less were considered statistically significant.

**Results**

**Subject characteristics**

Characteristics of patients and healthy subjects are summarised in ►Table 1. With respect to haematological findings, erythrocytes, haemoglobin and haematocrit in the PV or SP group were significantly higher than that in healthy group. In addition, platelet, leucocyte and neutrophil counts in PV group were significantly higher than that in the SP group or healthy group (►Table 1).

**Number and cellular origin of MPs**

The total number of MPs (i.e. lactadherin-positive particles) was significantly higher (4,589 ± 1,006/µl) in PV patients than that in SP patients (1,458 ± 802/µl; p<0.001 vs PV patients) or healthy controls (1,554 ± 643/µl; p<0.001 vs PV patients). However, there was no difference in total MP number between SP patients and healthy controls (p=0.148) (►Figure 1A).

The phenotypic characterisation of MPs in PV patients showed an increase in lactadherin⁺ MPs, which were mostly originated...
Similar to the total MP number, there was also no significant difference on MP number of each cellular type between SP patients and healthy controls. In addition, the correlation between MP number and platelet, erythrocyte, neutrophil counts showed that MP level was not related to their original cell numbers (Table 2).

**PS exposure on erythrocyte and platelet membranes**

Based on the results from the MPs analysis, we further measured the extent of PS exposure of erythrocytes and platelets by flow cytometry. As shown in Figure 2, PS exposure on the erythrocyte or platelet membranes in patients with PV was 3.58 ± 0.96% and 4.75 ± 1.24%, respectively, which was significantly higher compared to patients with SP (erythrocyte: 0.53 ± 0.24%, p<0.001; platelet: 0.94 ± 0.35%, p<0.01) or healthy subjects (erythrocyte: 0.55 ± 0.11%, p<0.001; platelet: 0.86 ± 0.23%, p<0.01). Since erythrocyte and platelet counts were higher in the PV group compared to the SP group or healthy group (Table 1), the significantly increased percentages of PS+ erythrocytes/platelets also means the significantly increased absolute numbers of these PS+ cells in PV patients.

**PCA of PV MPs, erythrocytes and platelets**

To explore the contribution of PS externalisation to hypercoagulable state in PV patients, PCA of MPs/erythrocytes/platelets from healthy individuals and patients was assessed by recalcification-time assays. The results showed that MPs/platelets/erythrocytes isolated from PV patients had markedly shortened clotting time (p<0.01), compared with equal volumes/numbers of MPs/cells from SP patients or control subjects (Figure 3A). To confirm

![Figure 2: Flow cytometry analyses of PS exposure on erythrocytes/platelets.](image-url)

![Figure 3: Procoagulant activity (PCA) of MPs/platelets/erythrocytes.](image-url)
that the detected PCA of MPs/erythrocytes/platelets in PV patients was related to PS or to TF, we performed coagulation inhibition assays. PS and TF were blocked with lactadherin and anti-TF antibody individually. Results showed that the coagulation times of PV MPs/erythrocytes/platelets were prolonged to control level by 128 nM lactadherin, while 40-μg/ml anti-TF did not affect the coagulation times significantly (Figure 4A). The PCA of MPs/erythrocytes/platelets was further analysed in FXase and prothrombinase assays. Our results showed that, with identical numbers of erythrocytes/platelets or with identical volume of MP suspension, intrinsic FXase complex and thrombin generation was significant higher in PV patients (p<0.01) than that in SP patients and control subjects (Figure 4B-C). However, MPs and cells prepared from either patients or healthy controls supported an extremely small amount of extrinsic FXase generation (data not shown). Inhibition assays of intrinsic FXase complex and thrombin formation were also performed. Data were normalised to facilitate comparison of the inhibition effect of lactadherin. We found that lactadherin inhibited the production of thrombin and intrinsic FXase complex in a dose-dependent manner, with about 90% of thrombin generation or intrinsic FXa complex production inhibited by 128 nM lactadherin (Figure 4B-C).

Influence of HU treatment on MPs release and PS exposure

To determine the influence of HU treatment on MP release and PS exposure, 12 PV patients provided blood sample before and after HU treatment. Before HU administration, the 12 patients showed significant higher circulating MPs and PS exposing erythrocytes/platelets than healthy subjects (p<0.01 for all). As expected, HU treatment induced a significant decrease (p<0.05 by paired test) of erythrocyte, platelet and neutrophil counts. In addition, MP release and PS exposure of erythrocytes/platelets significantly declined after HU therapy. The level of MPs from granulocytes and endothelial cells were not significantly different (Table 3). However, the MP release and PS exposure of erythrocytes/platelets was still significantly higher in HU-treated PV patients than that in healthy subjects (p<0.05 for all).

Discussion

In the present study, we demonstrated that PV patients had elevated numbers of PS MPs in their circulation, originating from erythrocytes, platelets, granulocytes and endothelial cells. Meanwhile, PS exposing erythrocytes/platelets were significantly elevated. More important, we demonstrated here that PS exposure on MPs/erythrocytes/platelets in PV patients was highly procoagulant as confirmed by shortened clotting time and support of the intrinsic FXase and prothrombinase complexes. Moreover, blockade of PS with lactadherin effectively inhibited activity of procoagulant enzyme complexes and the consequent PCA of MPs/erythrocytes/platelets.
platelets. Our findings demonstrate that the hypercoagulability of PV can be attributed to the increased level of PS+ MPs/erythrocytes/platelets in the circulation.

Fujita et al. recently reported that percentage of PS+ erythrocytes/platelets was higher in PV patients than in healthy subjects (16, 17). However, these authors did not measure their PCA, nor did they determine the MP release by these cells. In addition, using functional assays to analyse the increase of blood MPs cannot distinguish MPs from different sources and may also detect activated platelets and large apoptotic bodies (29). Our present results extended these findings and demonstrated, for the first time, that patients with PV had elevated levels of blood MPs of erythrocyte, platelet, granulocyte and endothelial cell origin. We also confirmed that MPs/erythrocytes/platelets in PV patients were potently procoagulant, owing to the abnormal express of PS on their outer leaflets. PS exposure and MP release indicate cell activation, injury or possibly apoptosis (10). We confirmed findings by others (6, 30, 31) that platelets/endothelial cells/granulocytes were activated and erythrocyte deformability was decrease in patients with PV.

In PV patients, erythrocytosis is the most prominent clinical feature and the "sine qua non" for its diagnosis (1). In present study, levels of MPs and extent of PS exposure on erythrocytes and platelets were also investigated in patients with SP. Our data proved that no significant differences were observed between SP patients and healthy subjects in MP cellular-origin distribution and in PS exposure on erythrocytes/platelets. Since erythrocytosis, higher haematocrit values and hyperviscosity may also be present in SP patients, we therefore conclude that increased MP generation and PS exposure are not simply attributable to elevated erythrocyte counts, higher haematocrit values or hyperviscosity. In addition, neither in polycythaemia patients nor in healthy subjects did MP levels relate to their original cell numbers. These data suggest that MP formation may be a regulated rather than a constitutive process. Our data may provide a new clue for separating PV from SP.

HU is an antimetabolite that introduced in the therapy of PV by the Polycythemia Vera Study Group (PVSG). The aim of HU therapy is to control myeloproliferation and avoid vascular complications. In the present study, we found that treatment with HU lowered MP release and PS exposure of erythrocytes/platelets. This effect of HU is also confirmed in patients with sickle cell anaemia (32, 33). However, Trappenburg et al. found no effect of HU on the number of MPs in patients with essential thrombocythemia (34). This result is not in contradiction with our observation because they compared seven HU-treated patients to the remaining aspirin-treated or untreated patients, while paired sample analysis was used in our study. Also, the number of their patients may not have been large enough to evidence any influence of the HU treatment. Our data may represent a novel target for the antithrombotic activity of HU in PV. The mechanism responsible for decrease of MP release and PS exposure in HU as well as its discrepancy effect on different cell types require further study and elucidation. Moreover, our results lead to believe that certain factors specific for PV itself contribute to the increased MPs release and PS exposure.

A number of methods are applied for MP detection. Yuana et al. have recently described the strengths and weaknesses of the different techniques to measure MPs (35). Among these techniques, flow cytometry is the most widely applied technique for MP analysis. Number and origin abnormalities of MPs in particular size distribution can prompt clinical or pathogenetic significance. Recently, the introduction of TrueCount beads and fluorescent calibrated sub-micrometer beads allows standardization and repeatability of the MP quantification by flow cytometry (27). Fluorescence-nanoparticle tracking analysis and atomic force microscopy may be used to count small MPs, but the two methods are still in their infancy and they are not suitable for direct quantification of MPs in plasma. Meanwhile atomic force microscopy is time consuming and how to attach all plasma MPs on a mica surface is a question to be addressed. In addition, pre-analytical variables are also important factors affecting MP analysis (35). Therefore, we believe that further efforts are necessary to standardize protocols and methods for MP assessment.

Circulating MPs are important procoagulant factors involved in hypercoagulability (15, 36) and are thought to be involved in the pathogenesis of cancer-associated thrombosis (37, 38). In present study, PS-dependent procoagulant activity of PV MPs/erythrocytes/platelets was measured. We demonstrated that MPs/erythrocytes/platelets prepared from patients with PV promoted the coagulation cascade reaction by providing PS for assembly of the FXase and prothrombinase complexes, and subsequently lead to a dramatic increase in thrombin generation. To distinguish the role of PS and TF on the procoagulant activity of MPs/erythrocytes/platelets, inhibition assays were performed. We proved that blockade of PS with lactadherin restored coagulation times of MPs/erythrocytes/platelets to control levels. In addition, lactadherin inhibited about 90% of the FXa and thrombin formation in a dose-response manner. However, anti-TF antibody did not affect the procoagulant activity of MPs/erythrocytes/platelets. Accounting for this are the findings by a previous study that erythrocytes do not express TF, and platelet expression of TF is still uncertain (39). Moreover, MPs/erythrocytes/platelets supported an extremely procoagulant activity of MPs/erythrocytes/platelets.

What is known about this topic?
- Patients with polycythaemia vera (PV) are characterised by increased circulating procoagulant microparticles (MPs) and phosphatidylserine (PS) exposing erythrocytes/platelets.
- The hypercoagulable state in PV is partially explained by the increased PS+ MPs originated from secondary polycythaemia patients may provide a new clue for separating PV from secondary polycythaemia.
- Treatment with hydroxyurea lowers MP release and PS exposure on erythrocytes and platelets, which may represent a novel target for the antithrombotic activity of hydroxyurea in PV.

What does this paper add?
- The total number of circulating PS+ MPs in PV patients is augmented, mostly originating from platelets, erythrocytes, granulocytes and endothelial cells.
- Significant differences in MP levels between PV and secondary polycythaemia patients may provide a new clue for separating PV from secondary polycythaemia.

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small amount of extrinsic FXase generation and few MPs exposing TF were detected in our study. Therefore, strong evidence demonstrates that augmented procoagulant activity of MPs/erythrocytes/platelets is principally PS-dependent. Our study suggests that PS exposure on the outer membrane leaflets of MP/erythrocytes/platelets is highly procoagulant, which may be a factor inducing a hypercoagulable state in PV.

In conclusion, we demonstrate that PV patients are characterised by increased circulating PS MPs/erythrocytes/platelets. These PS-bearing MPs and cells are highly procoagulant and could, therefore, contribute to the hypercoagulable state in PV. The present study also documents that treatment with HU is associated with a decrease in the lactadherin MP release of erythrocytes/platelets. Further study is strongly warranted to clear the prognostic role of PS exposure and MP generation for thromboembolic complications in PV.

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Conflicts of interest
None declared.

References