

Distribution characteristics of cells in splenomegaly due to hepatitis B-related cirrhotic portal hypertension and their clinical importance

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Abstract

Objectives: To investigate peripheral cytopenia in patients with splenomegaly due to hepatitis B-related cirrhotic portal hypertension (HBRCPH) by comparing blood cell counts from enlarged spleens with peripheral blood.

Methods: This prospective study involved patients undergoing splenectomy at the Nangfang Hospital from June 2013 to December 2015. Blood cell counts from peripheral blood were compared with those from splenic blood taken during splenectomies.

Results: Clinical data were available from 30 patients. White blood cell (WBC), red blood cell (RBC) and platelet counts were statistically significantly lower in peripheral blood compared with splenic blood. After splenectomy, peripheral blood cell counts increased significantly compared with pre-operative levels. Platelet and WBC counts in the lower spleen were significantly higher than those in the porta lienis (middle segment) and upper spleen.

Conclusions: In patients with splenomegaly due to HBRCPH, the counts of three blood cell lineages were significantly higher in the spleen than in peripheral blood. Splenectomy can aid the return of peripheral blood cell counts to normal levels. The most significant retention of platelets and WBCs occurred in the lower spleen which may be useful information for surgeons performing partial splenectomies.

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Keywords

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Introduction

In China, cirrhosis is relatively common disease and is due primarily to viral hepatitis.¹ Generally, the early clinical manifestation is liver dysfunction but portal hypertension can occur during the later stages of cirrhosis.² Cirrhosis is often complicated by peripheral cytopenia of single or multiple lineages and a prevalence of 90% has been reported.³ Moreover, the greater the number of lineages involved in cytopenia, the worse is the prognosis.⁴ Peripheral cytopenia, defined as a peripheral white blood cell (WBC) count $<4.0 \times 10^9/l$, red blood cell (RBC) count $<3.5 \times 10^{12}/l$, or platelet count $<100 \times 10^9/l$ can be life-threatening and so investigating its cause is crucial.^{1,5}

Peripheral cytopenia due to cirrhotic portal hypertension may be caused by several factors.⁶ In the past, researchers proposed a link between leukopenia and hypersplenism⁷ and suggested diagnostic criteria for hypersplenism, the first of which was the presence of single-lineage or multiple-lineage peripheral cytopenia.⁸ In recent years, investigators have reported that infection of hematopoietic stem cells within bone marrow by hepatitis viruses,⁹ dysfunction of endothelial cells within bone marrow,¹⁰ liver dysfunction,^{11,12} and excessive drug toxicity (e.g., from cyclophosphamide)¹³ can also cause peripheral cytopenia. Nevertheless, splenomegaly and cell retention in the spleen have been suggested by several researchers as the primary cause of peripheral cytopenia.^{14,15} However, evidence to support the link has never been proven.¹⁶

In this pilot study involving patients with splenomegaly due to post-hepatic cirrhotic portal hypertension, we compared blood cell counts from the spleen with those from peripheral blood. The intention was to use the data from this study to inform the design of a larger study into the role of peripheral cytopenia in post-hepatic cirrhotic portal hypertension.

Patients and methods

This prospective study involved patients undergoing splenectomies at the Nangfang Hospital (Guangzhou, China) from June 2013 to December 2015. The study was approved by the hospital ethics committee (Ethical approval No.: Med-Eth-Re [2017] 44) and informed consent was obtained from all patients. Eligible patients had hepatitis B-related cirrhosis, a significantly enlarged spleen confirmed by B-mode ultrasound or computed tomography (CT), blood cell count data (peripheral and splenic) and a successful surgical outcome.

For peripheral blood, a 2 ml sample was collected following an overnight fast before and after surgery and placed in a blood-collection tube containing K2-EDTA (VacutainerTM; Becton Dickinson, Franklin Lakes, NJ, USA). The post-operative sample was taken just prior to discharge from hospital. Blood from the spleen was taken during the splenectomy. The resected spleen was divided into three segments (upper, porta lienis [middle] and lower) (Figure 1); each segment was incised rapidly at its centre with a scalpel and approximately 1.5 ml of venous blood was

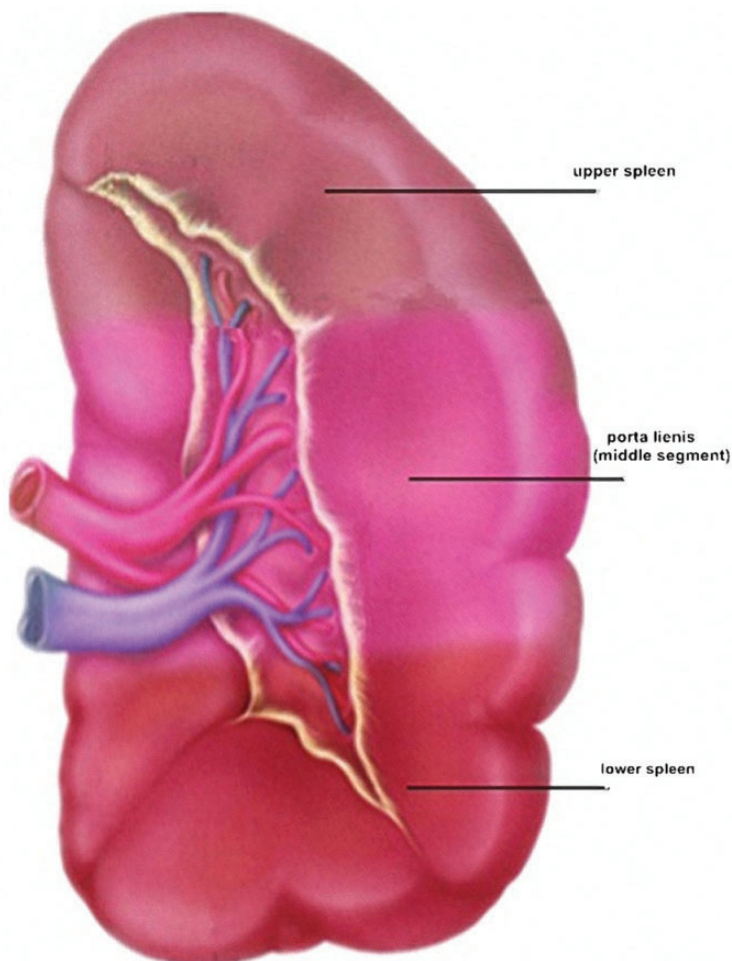


Figure 1. The resected spleen was divided into three segments (upper, porta lienis [middle] and lower).

collected from the incision and placed in a K2-EDTA tube (Figure 2). All blood samples were mixed thoroughly and analysed by the hospital laboratory within 2h at room temperature using the SysmexXE-2100 automated blood cell counter (Sysmex, Kobe, Japan). Blood samples and data extraction were performed by two investigators (X.H. and N.L.)

Statistical analyses

Statistical analyses were performed by two investigators (Y.L. and J.D.) using SPSS

software (version 19.0 for Windows®; IBM SPSS, Armonk, NY: IBM Corp, USA). A P -value <0.05 was considered to indicate statistical significance.

Continuous variables were expressed as mean \pm standard deviation (SD). Splenic and peripheral blood cells were compared using a paired-sample t -tests. The three blood cell lineages from the three segments of the enlarged spleen were compared using a one-way analysis of variance (ANOVA). A *post hoc* test was performed using a Bonferroni adjustment.

Correlations between splenic and peripheral blood cells were analysed using Pearson's correlation analysis.

Results

Thirty patients (24 males and 6 females; age, 41.0 ± 20.0 years; age range 21–61 years) were eligible for the study. All patients had a history of bleeding within the upper gastrointestinal tract and had a significantly enlarged spleen with a central thickness of 71.5 ± 17.6 (range 54–88) mm and a vertical diameter of 188 ± 48.0 (range 140–236) mm. Nineteen patients had been



Figure 2. Blood collection following an incision at different regions of the spleen.

hospitalized due to massive variceal haemorrhage in the oesophagus and gastric fundus and underwent splenectomy and pericardial devascularization. The remaining 11 patients had undergone splenectomy for moderate/severe hypersplenism.⁴ Gastroscopy showed moderate and severe varicose veins in the lower oesophagus and gastric fundus in all patients. Eight patients had single-lineage peripheral cytopenia and 22 had multiple-lineage peripheral cytopenia. Cirrhotic nodules on the liver surface, congestive enlargement of the spleen and 20–500 ml of ascites were observed intra-operatively. Following the surgery, all patients were discharged from hospital.

Statistically significant differences between splenic and peripheral blood cell counts were observed in terms of WBC ($P < 0.0001$), RBC ($P = 0.031$) and platelets ($P < 0.0001$) (Table 1). Platelet, WBC and RBC counts in the enlarged spleen were approximately 2.83-, 2.76- and 1.30- times that in the peripheral blood, respectively. In addition, as shown by pre- and post-operative peripheral blood cell counts, the counts of all three blood cell lineages were increased significantly after splenectomy (Table 2).

No differences were detected in RBC counts from different segments of the spleen (i.e., upper, porta lienis, or lower spleen). However, statistically significant differences in WBC and platelet counts

Table 1. Comparison of blood cell counts from enlarged spleens and peripheral blood taken from patients undergoing splenectomy due to hepatitis B-related cirrhotic portal hypertension.

	Splenic Blood <i>n</i> =30	Peripheral Blood* (pre-operative) <i>n</i> =30	Statistical significance
White blood cells, $\times 10^9/l$	10.6 ± 4.8	3.8 ± 1.6	$P < 0.0001$
Red blood cells, $\times 10^{12}/l$	3.9 ± 1.4	3.0 ± 0.6	$P = 0.031$
Platelets, $\times 10^9/l$	170.0 ± 65.7	60.0 ± 26.5	$P < 0.0001$

Values are shown as mean \pm standard deviation

*Peripheral cytopenia is defined as a peripheral white blood cell count $< 4.0 \times 10^9/l$, red blood cell count $< 3.5 \times 10^{12}/l$, or platelet count $< 100 \times 10^9/l$.¹

Table 2. Comparison of pre-operative and post-operative blood cell counts in peripheral blood taken from patients undergoing splenectomy due to hepatitis B-related cirrhotic portal hypertension.

	Pre-operative blood sample* n=30	Post-operative blood sample n=30	Statistical significance
White blood cells, $\times 10^9/l$	4.2 \pm 1.7	12.6 \pm 7.4	$P < 0.0001$
Red blood cells, $\times 10^{12}/l$	3.1 \pm 0.5	3.9 \pm 1.0	$P = 0.002$
Platelets, $\times 10^9/l$	62.7 \pm 53.3	211.2 \pm 138.4	$P < 0.0001$

Values are shown as mean \pm standard deviation

*Peripheral cytopenia is defined as a peripheral white blood cell count $< 4.0 \times 10^9/l$, red blood cell count $< 3.5 \times 10^{12}/l$, or platelet count $< 100 \times 10^9/l$.¹

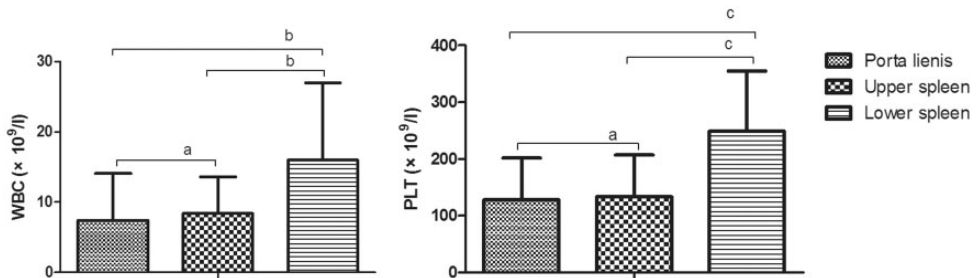


Figure 3. Comparison of white blood cells (WBC) and platelets (PLT) among different areas of the enlarged spleen (^anot statistically significant; ^b $P < 0.05$; ^c $P < 0.01$). There were no differences in red blood cell counts (data not shown).

were observed among the three segments of the enlarged spleen (Figure 3). A diagrammatic representation of the blood cell distribution in the enlarged spleen and peripheral blood is shown in Figure 4.

There was no correlation between enlarged spleen or peripheral blood counts in terms of WBC or RBC (Pearson correlation analysis). However, there was a positive correlation between platelet counts in the enlarged spleen and peripheral blood ($r = 0.64$, $P = 0.014$).

Discussion

In this study, the counts of all three blood cell lineages were significantly higher in the enlarged spleen compared with the peripheral blood which was probably due to the retention of blood cells in the spleen and

hypersplenism.¹⁷ In cirrhotic portal hypertension, blood is re-allocated to the spleen, which leads to hyperaemic splenomegaly and spleen size can be increased up to 8–10-times that of a normal spleen.¹⁷ As the condition progresses, the blood pool within the spleen increases significantly, allowing more and more blood cells to be stored.¹⁸ Blood cells retained in the enlarged spleen can be captured, phagocytosed, or destroyed by the active monocyte–macrophage system. Indeed, the phagocytic activity of macrophages in the spleen has been shown to increase with splenomegaly. The increased number and activity of macrophages in the spleen may be important causes of peripheral cytopenias due to splenomegaly.² In this present study, the counts of RBCs, WBCs and platelets in the peripheral blood increased

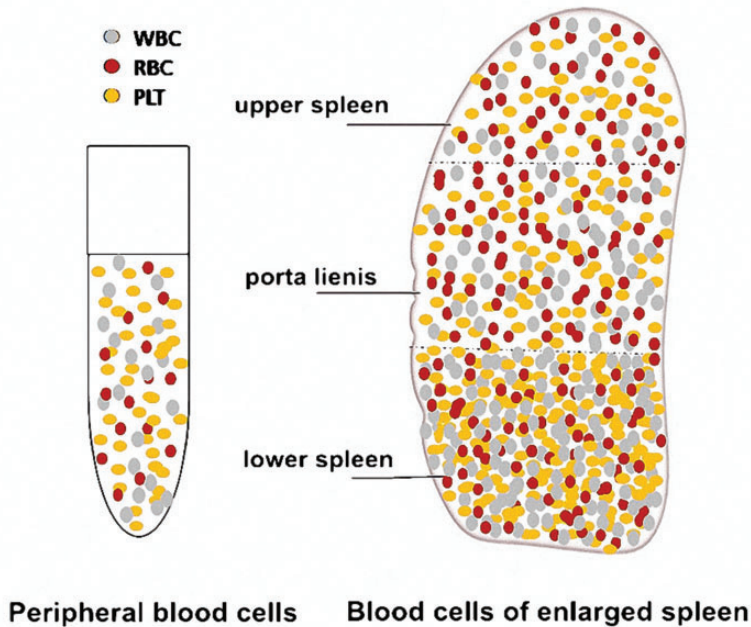


Figure 4. A diagrammatic representation of the blood cell distribution in the enlarged spleen and peripheral blood.

significantly following splenectomy because the site of blood cell retention had been removed.¹⁹

Our study showed that blood cells were distributed unevenly in the enlarged spleen. Although there was no difference in RBC counts, platelet and WBC counts were significantly lower in the lower spleen compared with the porta lienis (middle) or upper spleen. A potential explanation for this could be the lower anatomic position of the lower spleen which may have facilitated the accumulation of platelets and WBCs. The distribution pattern of platelets and WBCs which we observed in the enlarged spleen may be useful information for surgeons when performing partial splenectomies.

Although there was no correlation between WBC and RBC counts in the enlarged spleen with those in peripheral blood, there was a significant correlation

between the platelet counts. These findings suggest that platelets in the enlarged spleen have a significant influence on peripheral platelets. Using ⁵¹Cr-labeled platelets, researchers showed that healthy individuals store one-third of the platelet count in the spleen with the remaining two-thirds stored in the peripheral circulation.¹⁸ However, 50–90% of platelets are retained in an enlarged spleen during hypersplenism, resulting in a reduction in platelet numbers in the circulating blood. One study found a significant correlation between thrombocytopenia and aetiology of cirrhosis.²⁰ In addition, another study showed that platelet distribution returned to normal after splenectomy,²¹ which likely eliminated platelet retention by the splenic blood pool and removed the site of hypersplenism. However, peripheral cytopenia in hepatitis B-related cirrhotic portal hypertension are thought to be caused by several factors.⁶

Therefore, although theories of splenic blood cell retention and hypersplenism may be valid, they do not explain the observation that reduced blood cell counts do not return to normal after splenectomy in a small number of patients.¹

Limitations of the study include its small sample size and non-comparative design. However, this was a pilot study and data were only available from patients undergoing splenectomies. Moreover, to our knowledge this is the first study to show that in patients with splenomegaly due to hepatitis and cirrhotic portal hypertension, the counts of the three blood cell lineages were significantly higher in the spleen than in peripheral blood. In addition, the study also showed the distribution characteristics of the three blood cell lineages in the enlarged spleen. Importantly, the most significant retention of platelets and WBCs occurred in the lower spleen. This finding may be of use to surgeons performing partial splenectomies.

Declaration of conflicting interests

The authors declare that there are no conflicts of interest.

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