Study regarding the regenerative response after induced anemia through blood collection in mouse

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Abstract
Ordinarily, animals can tolerate a loss of about 20 to 30% of the total quantity of blood without the need for transfusions or fluid therapy. Regarding mice, it is generally accepted that blood samples should be limited to 10 – 15% of total blood mass, in order to prevent the onset of anemia. For this experiment we have shown the hematological differences between the collection of 25% and 40% of total estimated blood volume. In both cases, complete blood counts were analyzed and erythropoietic response was described by means of bone marrow and spleen cytology analysis. After approximately 24 hours, both groups had increased reticulocyte counts and low hemoglobin levels. Red blood cell morphology was not altered with the exception of anisocytosis due to polychromasia.
***Keywords**: erythropoiesis, mouse, regenerative anemia*

Introduction

In the mouse, blood sample quantity should be carefully assessed. Samples larger than 10% to 20% of total blood volume may lead to the onset of anemia and can cause a series of pathophysiological effects on the animal (3) and may require fluid replacement (2, 4). Severe blood loss can cause hypovolemic shock, resulting in the death.

Acute anemia is a consequence of a loss of massive quantities of blood and is usually associated with bleeding from trauma or neoplasia, and hemolysis frequently due to toxic agents, drugs or autoimmune diseases (1).

Acute blood loss anemia is associated with a regenerative response, proportional to its severity. It is normally characterized by a decreased number of red blood cells (RBC) and packed cell volume (PCV). The anemia is usually normochromic, normocytic. Erythropoiesis in the mouse is evaluated by assessing bone marrow and spleen cellularity (6). The spleen remains a constant hematopoietic site throughout the animal's lifetime (7).

The aim of this study was to show the effects of removing 25% and 40% of total estimated blood volume by observing the regenerative response 48 hours after blood collection.

Materials and methods

Fifteen 10-week-old CD1 albino female mice were used in this experiment. The mice were quarantined for seven days for acclimatization and then were divided into three equal groups, respectively, two experimental groups and a control group.

All mice were fed a specific rodent diet and had free access to water. Evey mouse was weighed and the necesary amount of blood was gathered by the tail vessel snip techinque. Estimated total blood volume was calculated for each mouse using body weight measurements and the average blood volume in the body (8% of total body mass). The first group (Group A) was bled of 25% of total estimated blood volume and the second group (Group B) of 40% of total estimated blood volume.

After two days, the mice were anesthetized with ether, and blood samples were collected by cardiocentesis as a terminal procedure. Blood was collected in EDTA-coated microtainers.

Complete blood count (CBC) was obtained with an automated counting aparatus (Advia 2120i, Siemens).

Blood smears were made for the evaluation of peripheral blood cell cytology. Bone marrow smears were prepared from the femur using the ‚paintbrush' technique. The smears were made in the first 30 minutes after euthanasia. The myelogram was used in order to assess local cell morphology and M:E ratio (myeloid:erythroid).

Spleen impression smears were made in order to assess local cell morphology. Blood, bone marrow, and spleen smears were all stained using May-Grünwald Giemsa.

Results were statistically analysed using the GraphPad Prism 8 program. Mean and Standard deviations were calculated and the difference between the two experimental groups was shown using the paired t-Student test.

Results and discussion

The complete blood count showed modified paramethers, suggestive of regenerative anemia. Values were listed in Table 1. Both Group A and Group B had the same modified parameters.

There was a significant decrease of the number of RBCs of 6.98±0.71 $x10^6/\mu L$ for Group A, and 4.75±0.84 $x10^6/\mu L$ for group B. The amount of hemoglobin was also reduced, making the distinction of hypochromic anemia, with values of 11.3±0.4 g/dL for Group A and 6.8±0.7 g/dL for group B.

Packed cell volume was significantly modified in both groups, with mean values of 40.8±2.8% for group A and 25.5±4.5 for Group B. Slight decreases of the mean cell hemoglobin and mean cell hemoglobin concentration were noted in both groups.

Table 1. Complete blood count of the experimental and control groups

Parameter	Measuring unit	25% blood loss	40% blood loss	Control group
Red blood cells	$x10^6/\mu L$	6.98±0.71*	4.75±0.84*	9.56±0.63
Hemoglobin	g/dL	11.3±0.4*	6.8±0.7*	14.9±0.5
Packed cell volume	%	40.8±2.8*	25.5±4.5*	53.2±2.9
Mean cell volume	fL	58.6±2.8	54.5±3.2	56.2±3.7
Mean cell hemoglobin	pg	16.2±0.3	14.3±1.1	15.7±1.3
Mean cell hemoglobin concentration	g/dL	27.7±0.6	26.2±1.9	28.2±2.3
Red cell distribution width	%	17.8±1.5*	19.4±2.3*	12.7±0.3
Reticulocytes	$x10^9/L$	593.2±184	486.2±74.6	465.5±69.1
Platelets	$x10^3/\mu L$	1265±271	1471±195	1128±188
White blood cells	$x10^3/\mu L$	1.92±0.42	1.77±0.59	3.61±0.56

Data represents the mean value ± standard deviation calculated for 5 samples from each group.

**p<0.001*

There was a significant increase of the red cell distribution width values of 17.8±1.5% in group A and 19.4±2.3% in group B. There was a small increase of reticulocyte numbers in both groups, but the regenerative response is not proportional to the severity of the anemia.

At 48 hours post-bleeding, the regenerative response is visible in the complete blood count by means of reticulocyte number and red cell distribution width. Given the fact that most common hematology analyzers do not have reticulocyte counts or red cell distribution width as parameters, and the mean cell volume was not significantly modified as to provide a clear picture of cell size, the regenerative response may be easily overlooked at this stage.

Group A was characterized by mild regenerative anemia, whereas Group B had moderate regenerative anemia, but with a disproportional regenerative response at 48 hours post-bleeding.

Although we have found a study which states that it is safe to collect up to 25% of estimated total blood volume from female mice, without the onset of clinically significant anemia (5), the mice used in group A of this study have shown signs of mild regenerative anemia after 48 hours from sample collection. Although mild anemia is not life-threatening for the animal, the altered CBC could potentially influence study results.

Blood smear analysis has shown slight differences between the two groups, indicative of mild anemia, with anisocytosis (reflected in the CBC by the increase of red cell distribution width).

Group A presented mild polychromasia, erythrocytes were normocytic, normochromic without the presence of nucleated RBCs or Howell-Jolly bodies (Figure 1A). Group B has shown more marked polychromasia, with normocytic RBCs. As in the first group's case, there were no nucleated erythrocytes or Howell-Jolly bodies (Figure 1B).

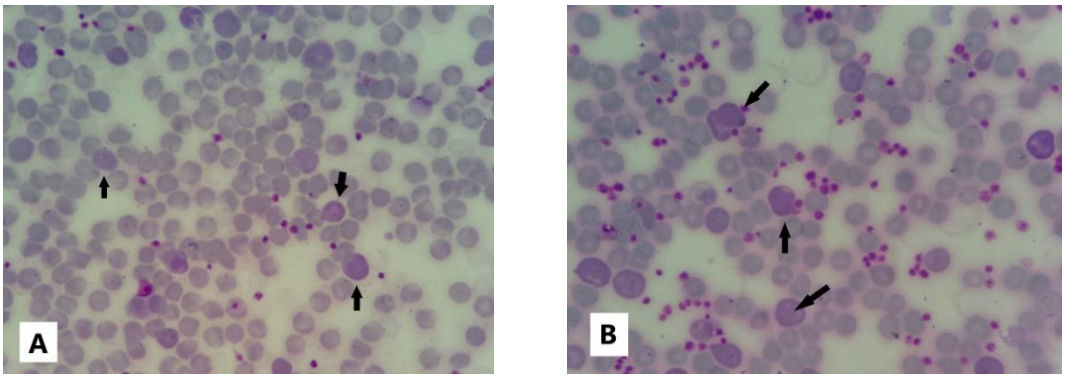

Figure 1. Blood smears images (100X). Group A with 25% blood loss shows signs of mild polychromasia with reticulocytes indicated by black arrows (A). Group B with 40% blood loss, with polychromasia and anisocytosis. Reticulocytes are indicated by black arrows. May-Grünwald Giemsa stain.
(Original images)

Bone marrow cytologic examination has shown normal cell morphology with a modified M:E ratio, due to erythroid hyperplasia, with a predominant population of rubricytes and metarubricytes (Figure 2A, 2B). The M:E ratio for the 25% blood loss group was of 1.5:1. The 40% blood loss group has shown a mean M:E ratio of 1.9:1. The control group had a mean M:E ratio of 2.1:1. Cell morphology and placement were normal.

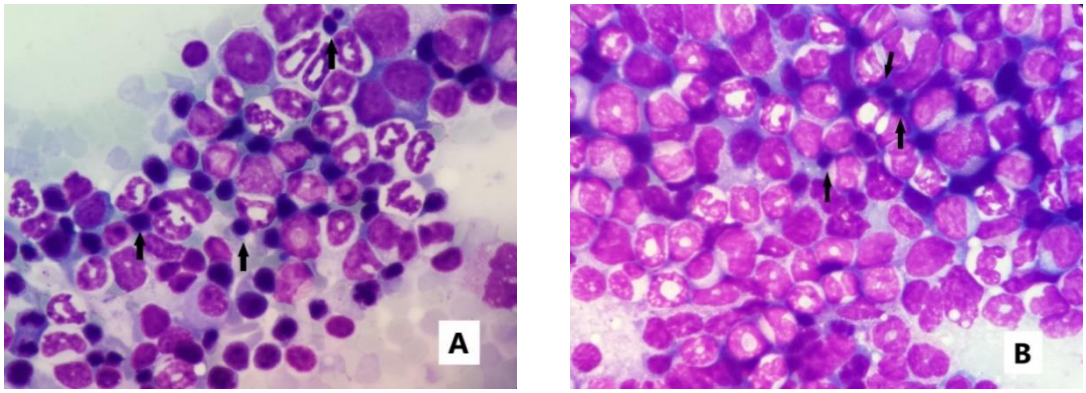

Figure 2. Bone marrow cytology (100x) with metarubricytes indicated by black arrows. A. Significant regenerative response observed in mouse group A, with the predominant cell line or late erythrocyte precursors such as rubricytes and metarubricytes. B. Bone marrow belonging to the 40 % blood loss group, with a slight regenerative response and a relatively high number of metarubricytes and rubricytes. May-Grünwald Giemsa stain.
(Original images)

Spleen macroscopic assessment has been made, and there were no visible lesions or changes in consistency and color. Organ weight was well within age and sex parameters. Spleen cytology has shown increased hematopoietic cellularity with marked erythroid hyperplasia with orderly maturation, which was more visible in Group B (Figure 3A, 3B).

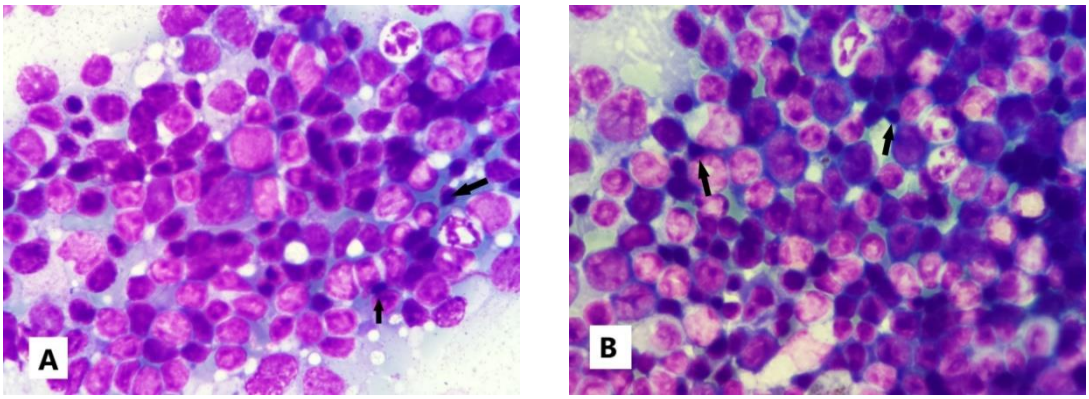

Figure 3. Spleen cytology (100x). Regenerative response observed in both experimental groups, with a predominant red blood cell progenitor population of metarubricytes (black arrows) and rubricytes. May-Grünwald Giemsa stain.
(Original images)

Bone marrow and spleen cytology confirmed the onset of a regenerative response, with a complete erythroid series, with orderly maturation. Erythroid hyperplasia was more intense in the first experimental group in the CBC results and bone marrow cytology, whereas the second experimental group had a more visible regenerative response of the splenic hematopoietic tissue.

Conclusion

During the first 48 hours after blood collection there were no major differences between the intensity of the regenerative response between the two experimental groups.

The intensity of the regenerative response was more obvious in spleen and bone marrow cytology than blood smear and hemogram.

Collecting an amount of blood of 25% or higher of estimated total blood volume leads to significant changes of the hemogram which can interfere with certain study results.

For a more clear understanding, this study will continue with observations of the regenerative response at 24 and 72 hours post-bleeding.

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