RETICULOCYTE COUNT TO ASSESS REGENERATIVE RESPONSE IN DOGS WITH IMMUNE MEDIATED HAEMOLYTIC ANAEMIA

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Abstract

Twenty five immune mediated haemolytic anaemia cases confirmed by saline agglutination test and Coombs' test were studied for bone marrow response on the day of presentation. Giemsa stained blood smears were examined to determine presence of any haemoparasites. Fresh whole blood sample collected were mixed with equal quantity of new methylene blue stain and smears were prepared. Reticulocyte count was estimated manually and expressed in percentage, absolute reticulocyte count and corrected reticulocyte percentage. About fifty six per cent of immune mediated haemolytic anaemia dogs showed regenerative anaemia on day of presentation. Results showed that immune mediated haemolytic anaemia had reticulocytosis and thus regenerative response. A reticulocyte count after five days of presentation is recommended to avoid false diagnosis of non-regenerative anemia and this may guide in considering pure red cell aplasia and non-regenerative immune mediated haemolytic anaemia in the differential diagnosis for immune mediated haemolytic anaemia.

Key Words: *Immune mediated haemolytic anaemia, new methylene blue stain, reticulocytosis*

Regenerative anemia results from haemolysis or acute blood loss such as haemorrhage, whereas chronic and inflammatory diseases like neoplasia, bone marrow disorders and iron deficiency can lead to non-regenerative anaemia. Early identification of type of anaemia is an essential diagnostic step aiding in further clinical evaluation and treatment (Cowgill *et al*., 2003). Reticulocytosis is considered as hallmark of increased erythropoiesis in the bone marrow and thus reticulocyte count is considered as gold standard test for assessing regenerative response (Hodges and Christopher, 2011).

Immune mediated haemolytic anaemia is a type II immunological response involving antibody mediated destruction of erythrocytes (Warman *et al*., 2008) resulting in severe anaemia with mean VPRC of 12 to 14 per cent (Piek, 2011). The objective of the study was to determine the regenerative response in IMHA dogs.

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Material and Methods

Twenty five dogs were diagnosed with immune mediated haemolytic anaemia (IMHA) by saline agglutination test and Coombs' test. Blood smear examination revealed presence of various haemoparasites such as *Babesia gibsoni*, *Ehrlichia canis*, *Mycoplasma haemocanis*, sheathed and unsheathed microfilariae. Reticulocyte count of IMHA dogs was estimated by manual method on the day of presentation and expressed in percentage, absolute reticulocyte count and corrected reticulocyte percentage to determine the regenerative response.

Manual method of reticulocyte count

Equal quantity of fresh EDTA-anticoagulated blood collected on day of presentation from IMHA dogs was mixed with equal quantity of new methylene blue stain (1% NMB in saline plus 1.6% potassium oxalate) and allowed to stand in the room temperature for 15 minutes. Smears were prepared after remixing the mixture. The number of reticulocytes were counted manually under oil immersion and expressed in percentage by evaluating 1000 non nucleated erythroids. Reticulocytes were also expressed as absolute reticulocyte count/µl by multiplying percentage of reticulocyte by the absolute erythrocyte count and corrected reticulocyte percentage.

$$\text{Corrected Reticulocyte percentage} = \frac{\text{(Calculated reticulocyte \% X Patient's VPRC)}}{\text{Average VPRC (0.45) for dogs}}$$

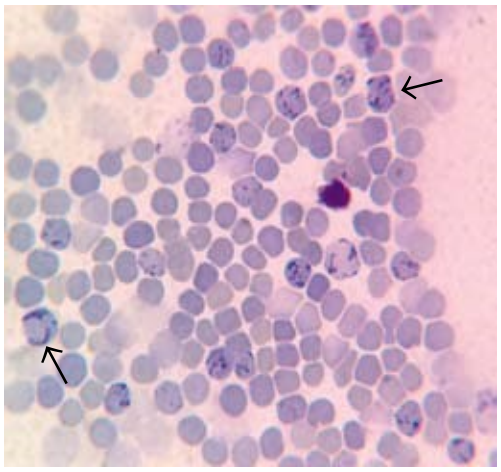

Fig. 1. New methylene blue stain demonstrating reticulocytosis in IMHA dogs (Black arrow)

Results and Discussion

Among the twenty five dogs, the absolute reticulocyte count was above 60000/µl in 13 dogs (52 per cent) and nine dogs had below 60000/µl (36 per cent). Corrected reticulocyte count was above one per cent in 14 dogs (56 per cent), 1 per cent in one dog (4 per cent) and less than one per cent in seven cases (28 per cent). Reticulocyte count was not estimated in three (12 per cent) cases due to death of dogs and non-availability of sample. The result is represented in table 1.

Table 1. Absolute reticulocyte count and Corrected reticulocyte percentage in IMHA dogs

Absolute Reticulocyte count		
Reference value	**Number**	**Per cent**
<60000/µl	9	36
>60000/µl	13	52
Not estimated	3	12
Corrected Reticulocyte percentage		
<1 per cent	7	28
1 per cent	1	4
>1 per cent	14	56
Not estimated	3	12
Total	25	100

In the present study absolute reticulocyte count of >60000/µl and corrected reticulocyte percentage of >1 per cent was recorded in 52 and 56 per cent of IMHA cases respectively indicating a regenerative type of anaemia (Balch and Mackin, 2007). The remaining dogs showed a non-regenerative anaemia which might be due to severity of the disease with insufficient time for mounting a regenerative response, as it takes four to five days for reticulocytosis to develop (Piek, 2011). Erythroid destruction could also be an important reason. In the present study all cases were diagnosed with secondary IMHA due to various haemoparasites that might result in improper bone marrow response, as suggested by Weinkle *et al*. (2005). If non regenerative anaemia persist pure red cell aplasia and non-regenerative IMHA should be considered in the differentials which demands bone marrow examination to confirm the diagnosis (Weiss, 2008). A reticulocyte count after five days

of diagnosis is recommended to avoid false diagnosis of non-regenerative anaemia and to confirm the type of anaemia.

To conclude, reticulocyte count can be good indicator of bone marrow response in IMHA dogs. The procedure has to be repeated after five days, if non regenerative anaemia is recorded on the day of presentation. Further this guides in considering pure red cell aplasia and non-regenerative IMHA in differential diagnosis.

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